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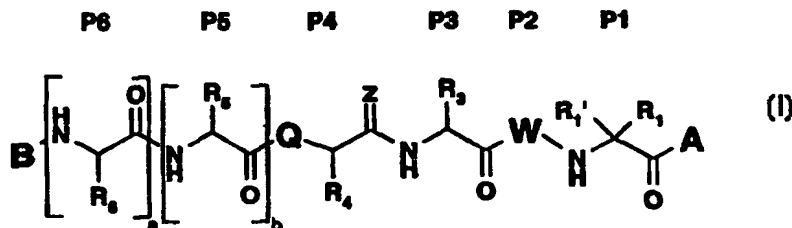
(54) Title: HEPATITIS C INHIBITOR PEPTIDES

(57) Abstract

Compound of formula (I) active against the Hepatitis C virus, wherein when Q is CH2, a is 0, b is 0 and B is an amide derivative; or when Q is N–Y wherein Y is H or C_{1-6} alkyl, then B is an acyl derivative; R_6, when present, is C_{1-6} alkyl substituted with carboxyl; R_5, when present, is C_{1-6} alkyl optionally substituted with carboxyl;

when Q is either CH2 or N–Y, then Z is oxo or thioxo; R_4 is C_{1-10}alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl); R_3 is C_{1-10} alkyl optionally substituted with carboxyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl); W is a proline derivative; R_1' is hydrogen, and R_1 is C_{1-6} alkyl optionnaly substituted with thiol; or R_1 is C_{2-6} alkenyl; or R_1' and R_1 together form a 3– to 6–membered ring; and A is hydroxy or a pharmaceutically acceptable salt or ester thereof.

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Hepatitis C Inhibitor Peptides

Field of the invention

5   The present invention relates to compounds,
     compositions and methods for the treatment of
     hepatitis C virus (HCV) infection. In particular,
     the present invention provides novel peptides and
     analogues thereof, pharmaceutical compositions
10   containing such peptides and methods for using these
     peptides in the treatment of HCV infection.

Background of the invention

15   Hepatitis C virus (HCV) is the major etiological
     agent of post-transfusion and community-acquired non-
     A non-B hepatitis worldwide. It is estimated that
     over 100 million people worldwide are infected by the
     virus. A high percentage of carriers become
20   chronically infected and many progress to chronic
     liver disease, so called chronic hepatitis C. This
     group is in turn at high risk for serious liver
     disease such as liver cirrhosis, hepatocellular
     carcinoma and terminal liver disease leading to
25   death.

     The mechanism by which HCV establishes viral
     persistence and causes a high rate of chronic liver
     disease has not been thoroughly elucidated. It is
30   not known how HCV interacts with and evades the host
     immune system. In addition, the roles of cellular
     and humoral immune responses in protection against
     HCV infection and disease have yet to be established.
     Immunoglobulins have been reported for prophylaxis of
35   transfusion-associated viral hepatitis. However, the

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Center for Disease Control does not presently
recommend immunoglobulins for this purpose.

5   The lack of an effective protective immune response
       is hampering the development of a vaccine or adequate
       post-exposure prophylaxis measures, so in the near-
       term, hopes are firmly pinned on antiviral
       interventions.

10   Various clinical studies have been conducted with the
       goal of identifying pharmaceutical agents capable of
       effectively treating HCV infection in patients
       afflicted with chronic hepatitis C. These studies
       have involved the use of interferon-alpha, alone and
15   in combination with other antiviral agents. Such
       studies have shown that a substantial number of the
       participants do not respond to these therapies, and
       of those that do respond favorably, a large
       proportion were found to relapse after termination of
20   treatment.

       Until recently, interferon (IFN) was the only
       available therapy of proven benefit approved in the
       clinic for patients with chronic hepatitis C. However
25   the sustained response rate is low, and interferon
       treatment also induces severe side-effects (i.e.
       retinopathy, thyroiditis, acute pancreatitis,
       depression) that diminish the quality of life of
       treated patients. Recently, interferon in
30   combination with ribavirin has been approved for
       patients non-responsive to IFN alone. However, the
       side effects caused by IFN are not alleviated with
       this combination therapy.

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Therefore, a need exists for the development of effective antiviral agents for treatment of HCV infection that overcomes the limitations of existing pharmaceutical therapies.

HCV is an enveloped positive strand RNA virus in the Flaviviridae family. The single strand HCV RNA genome is approximately 9500 nucleotides in length and has a single open reading frame (ORF) encoding a single large polyprotein of about 3000 amino acids. In infected cells, this polyprotein is cleaved at multiple sites by cellular and viral proteases to produce the structural and non-structural (NS) proteins. In the case of HCV, the generation of mature nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) is effected by two viral proteases. The first one, as yet poorly characterized, cleaves at the NS2-NS3 junction; the second one is a serine protease contained within the N-terminal region of NS3 (henceforth referred to as NS3 protease) and mediates all the subsequent cleavages downstream of NS3, both in *cis*, at the NS3-NS4A cleavage site, and in *trans*, for the remaining NS4A-NS4B, NS4B-NS5A, NS5A-NS5B sites. The NS4A protein appears to serve multiple functions, acting as a cofactor for the NS3 protease and possibly assisting in the membrane localization of NS3 and other viral replicase components. The complex formation of the NS3 protein with NS4A seems necessary to the processing events, enhancing the proteolytic efficiency at all of the sites. The NS3 protein also exhibits nucleoside triphosphatase and RNA helicase activities. NS5B is a RNA-dependent RNA polymerase that is involved in the replication of HCV.

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A general strategy for the development of antiviral
agents is to inactivate virally encoded enzymes that
are essential for the replication of the virus. In
5   this vein, patent application WO 97/06804 describes
the (-) enantiomer of the nucleoside analogue
cytosine-1,3-oxathiolane (also known as 3TC) as
active against HCV. This compound, although reported
as safe in previous clinical trials against HIV and
10   HBV, has yet to be clinically proven active against
HCV and its mechanism of action against the virus has
yet to be reported.

Intense efforts to discover compounds which inhibit
15   the NS3 protease or RNA helicase of HCV have led to
the following disclosures:

• US patent 5,633,388 describes heterocyclic-
   substituted carboxamides and analogues as being
20   active against HCV. These compounds are directed
   against the helicase activity of the NS3 protein
   of the virus but clinical tests have not yet been
   reported.

• A phenanthrenequinone has been reported by Chu *et*
25   *al* (Tet. Lett., (1996), 7229-7232) to have
   activity against the HCV NS3 protease *in vitro*.
   No further development on this compound has been
   reported.

• A paper presented at the Ninth International
30   Conference on Antiviral Research, Urabandai,
   Fukyshima, Japan (1996) (Antiviral Research, <u>30</u>,
   1, 1996; A23 (abstract 19)) reports thiazolidine
   derivatives to be inhibitory to the HCV protease.

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Several studies have reported compounds inhibitory to other serine proteases, such as human leukocyte elastase. One family of these compounds is reported in WO 95/33764 (Hoechst Marion Roussel, 1995). The peptides disclosed in that application are morpholinylcarbonyl-benzoyl-peptide analogues that are structurally different from the peptides of the present invention.

- WO 98/17679 from Vertex Pharmaceuticals Inc. discloses inhibitors of serine protease, particularly, Hepatitis C virus NS3 protease. These inhibitors are peptide analogues based on the NS5A/5B natural substrate that contain C-terminal activated carbonyl function as an essential feature. These peptides were also reported to be active against other serine protease and are therefore not specific for HCV NS3 protease.

- Hoffman LaRoche has also reported hexapeptides that are proteinase inhibitors useful as antiviral agents for the treatment of HCV infection. These peptides contain an aldehyde or a boronic acid at the C-terminus.

- Steinkühler et al. and Ingallinella et al. have published on NS4A-4B product inhibition (Biochemistry (1998), 37, 8899-8905 and 8906-8914). These peptides and peptide analogues were published after the priority date of the present application.

One advantage of the present invention is that it provides peptides that are inhibitory to the NS3 protease of the hepatitis C virus.

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A further advantage of one aspect of the present
invention resides in the fact that these peptides
specifically inhibit the NS3 protease and do not show
significant inhibitory activity at concentrations up
to 300 µM against other serine proteases such as
human leukocyte elastase (HLE), porcine pancreatic
elastase (PPE), or bovine pancreatic chymotrypsin, or
cysteine proteases such as human liver cathepsin B
(Cat B).

Summary of the invention

We investigated peptides potentially inhibitory to
the NS3 protease. The discovery that the N-terminal
cleavage product (**Ac-D-D-I-V-P-C-OH**) of an analogue
of a natural substrate of the NS3 protease was
inhibitory led us to the peptide analogues of the
present invention.

Included in the scope of the invention are compounds
of formula (I):

(I)
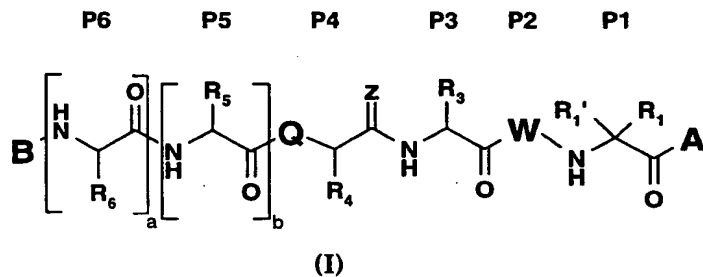
wherein **Q** is CH_2 or N-**Y** wherein **Y** is H or C_{1-6} alkyl;

a) when **Q** is CH_2, **a** is 0, **b** is 0, and **B** is an amide
derivative of formula $R_{11a}N(R_{11b})$-C(O)- wherein R_{11a} is
H; C_{1-10} alkyl; C_6 aryl; C_{7-10} alkylaryl; C_{3-7} cycloalkyl
optionally substituted with carboxyl; (C_{3-7}

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cycloalkyl)-(C_{1-6} alkyl); heterocycle-C_{1-6} alkyl such as

and R_{11b} is C_{1-6} alkyl substituted with carboxyl, (C_{1-6} alkoxy)carbonyl or phenylmethoxycarbonyl; or C_{7-16} aralkyl substituted on the aromatic portion with carboxyl, (C_{1-6} alkoxy)carbonyl or phenylmethoxycarbonyl;

or R_{11a} and R_{11b} are joined to form a 3 to 7-membered nitrogen-containing ring optionally substituted with carboxyl or (C_{1-6} alkoxy) carbonyl;
or

b) when Q is N-Y, a is 0 or 1, b is 0 or 1, and B is an acyl derivative of formula R_{11}-C(O)-wherein R_{11} is (i) C_{1-10} alkyl optionally substituted with carboxyl, C_{1-6} alkanoyloxy (e.g. $AcOCH_2$) or C_{1-6} alkoxy (e.g. Boc); (ii) C_{3-7} cycloalkyl optionally substituted with carboxyl, (C_{1-6} alkoxy)carbonyl or phenylmethoxycarbonyl; (iii) C_{3-7} cycloalkyl substituted with carboxyl and one to three C_{1-6} alkyl substituents (iv) C_{4-10} (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxy, (C_{1-6} alkoxy)carbonyl or phenylmethyoxycarbonyl; (v)

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(v) C_6 or C_{10} aryl or C_{7-16} aralkyl optionally
substituted with C_{1-6} alkyl;

R_6, when present, is C_{1-6} alkyl substituted with
carboxyl;

R_5, when present, is C_{1-6} alkyl optionally substituted
with carboxyl;

or

when Q is either CH_2 or $N-Y$;

c) R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}
(alkylcycloalkyl);

Z is oxo or thioxo;

R_3 is C_{1-10} alkyl optionally substituted with
carboxyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

W is a group of formula II:

Formula II

wherein R_2 is C_{1-10} alkyl or C_{3-10} cycloalkyl optionally
substituted with carboxyl; C_6 or C_{10} aryl or C_{7-16}
aralkyl; or

W is a group of formula II':

Formula II'

wherein X is CH or N; and

R_2' is divalent C_{3-4} alkylene which together with X
and the carbon atom to which X and R_2' are attached
form a 5- or 6-membered ring, said ring optionally
substituted with OH; SH; NH_2; carboxyl; R_{12}; OR_{12},
$C(O)OR_{12}$, SR_{12}, NHR_{12} or $NR_{12}R_{12}'$ wherein R_{12} and R_{12}' are
independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or
cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl,
said alkyl or alkenyl optionally substituted
with NH_2, OH, SH, halo, or carboxyl; said alkyl
5   or alkenyl optionally containing at least one
heteroatom selected independently from the group
consisting of: O, S, and N; or
R_{12} and R_{12}' are independently C_6 or C_{10} aryl or
C_{7-16} aralkyl optionally substituted with C_{1-6}
10   alkyl, CF_3, NH_2, OH, SH, halo, carboxyl, C_{1-6}
alkyl substituted with carboxyl or phenyl
optionally substituted with C_{1-6} alkyl, C_{1-6}
alkoxy, halo, acetylamido or nitro; said aryl or
aralkyl optionally containing at least one
15   heteroatom selected independently from the group
consisting of: O, S, and N;
said cyclic alkyl, cyclic alkenyl, aryl or
aralkyl being optionally fused with a second 5-,
6-, or 7-membered ring to form a cyclic system
20   or heterocyclic system, said second ring being
optionally substituted with NH_2, OH, SH, halo,
carboxyl or carboxy(lower)alkyl; said second
ring optionally containing at least one
heteroatom selected independently from the group
25   consisting of: O, S, and N;
or **X** is CH or N; and R_2, is a divalent C_{3-4} alkylene
which together with **X** and the carbon atom to which **X**
and R_2, are attached form a 5- or 6-membered ring
which in turn is fused with a second 5-, 6- or 7-
30   membered ring to form a cyclic system wherein the
second ring is substituted with OR_{12},, wherein R_{12},, is
C_{7-16} aralkyl;

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R_1' is hydrogen, and R_1 is C_{1-6} alkyl optionally
substituted with thiol or halo; or R_1 is C_{2-6} alkenyl;
or

R_1' and R_1 together form a 3- to 6-membered ring
5   optionally substituted with C_{1-6} alkyl; and
A is hydroxy or a pharmaceutically acceptable salt or
ester thereof.

Included within the scope of this invention is a
10   pharmaceutical composition comprising an anti-
hepatitis C virally effective amount of a compound of
formula I, or a therapeutically acceptable salt or
ester thereof, in admixture with a pharmaceutically
acceptable carrier medium or auxiliary agent.

15

An important aspect of the invention involves a
method of treating a hepatitis C viral infection in a
mammal by administering to the mammal an anti-
hepatitis C virally effective amount of the compound
20   of formula I, or a therapeutically acceptable salt or
ester thereof or a composition as described above.

Another important aspect involves a method of
inhibiting the replication of hepatitis C virus by
25   exposing the virus to a hepatitis C viral NS3
protease inhibiting amount of the compound of formula
I, or a therapeutically acceptable salt or ester
thereof or a composition as described above.

30   Still another aspect involves a method of treating a
hepatitis C viral infection in a mammal by
administering thereto an anti-hepatitis C virally
effective amount of a combination of the compound of
formula I, or a therapeutically acceptable salt or
35   ester thereof, and an interferon. A pharmaceutical

composition comprising the combination in admixture
with a pharmaceutically acceptable carrier medium or
auxiliary agent is also within the scope of this
invention.

5

Detailed description of the invention

As used herein, the following definitions apply
unless otherwise noted:

10

With reference to the instances where (R) or (S) is
used to designate the configuration of a radical,
e.g. R_4 of the compound of formula I, the designation
is done in the context of the compound and not in the
15   context of the radical alone.

The natural amino acids, with exception of glycine,
contain a chiral carbon atom. Unless otherwise
specifically indicated, the compounds containing
20   natural amino acids with the L-configuration are
preferred. However, applicants contemplate that when
specified, some amino acids of the formula I can be
of either D- or L- configuration or can be mixtures
of D- and L-isomers, including racemic mixtures.

25

The designation "P1, P2, P3 et." as used herein refer
to the position of the amino acid residues starting
from the C-terminus end of the peptide analogues and
extending towards the N-terminus (i.e. P1 refers to
30   position 1 from the C-terminus, P2: second position
from the C-terminus, etc.) (see Berger A. & Schechter
I., Transactions of the Royal Society London series
B257, 249-264 (1970)).

12

The abbreviations for the α-amino acids are set forth
in Table A.

Table A

AMINO ACID	SYMBOL
Allylglycine	AlGly
Aminobutyric acid	Abu
1-aminocyclopentyl-carboxylic acid	Acpe
1-aminocyclopropyl-carboxylic acid	Acca
Alanine	Ala
Aspartic acid	Asp
Cysteine	Cys
Cyclohexylalanine	Cha
Cyclohexylglycine (also named: 2-amino-2-cyclohexylacetic acid)	Chg
Glutamic acid	Glu
Isoleucine	Ile
Leucine	Leu
Norvaline	Nva
Phenylalanine	Phe
Pipecolic acid	Pip
Proline	Pro
4(R)-Hydroxyproline	Hyp
4(R)-Benzyloxyproline	Hyp(4-Bn)
Valine	Val
tert-Butylglycine	Tbg

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As used herein the term "aminobutyric acid" refers to
a compound of formula:

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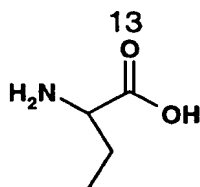
As used herein the term "allylglycine" refers to a compound of formula:

5
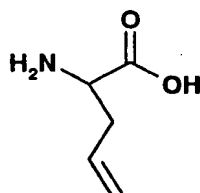
As used herein the term "1-aminocyclopropyl-carboxylic acid" (Acca) refers to a compound of formula:
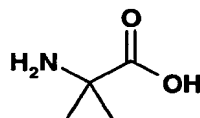
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As used herein the term "*tert*-butylglycine" refers to a compound of formula:
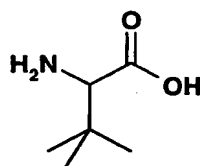
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The term "residue" with reference to an amino acid or amino acid derivative means a radical derived from the corresponding α-amino acid by eliminating the hydroxyl of the carboxy group and one hydrogen of the

20   α-amino group. For instance, the terms Gln, Ala, Gly, Ile, Arg, Asp, Phe, Ser, Leu, Cys, Asn, Sar and Tyr represent the "residues" of L-glutamine, L-alanine, glycine, L-isoleucine, L-arginine, L-aspartic acid,

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L-phenylalanine, L-serine, L-leucine, L-cysteine, L-
asparagine, sarcosine and L-tyrosine, respectively.

The term "side chain" with reference to an amino acid
5   or amino acid residue means a group attached to the
α-carbon atom of the α-amino acid. For example, the
R-group side chain for glycine is hydrogen, for
alanine it is methyl, for valine it is isopropyl.
For the specific R-groups or side chains of the α-
10   amino acids reference is made to A.L. Lehninger's
text on Biochemistry (see chapter 4).

The term "halo" as used herein means a halogen
radical selected from bromo, chloro, fluoro or iodo.
15
The term "C_{1-6} alkyl" or "(lower)alkyl" as used
herein, either alone or in combination with another
radical, means straight chain or branched alkyl
radicals containing up to six carbon atoms and
20   includes, for example, methyl, ethyl, propyl, butyl,
hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl,
1,1-dimethylethyl.

Likewise, the terms "C_{1-3} alkyl" "C_{1-4} alkyl" and "C_{1-10}
25   alkyl" are used to denote alkyl radials containing up
to three, four and ten carbon atoms, respectively.

The term "C_{3-7} cycloalkyl" as used herein, either
alone or in combination with another radical, means a
30   cycloalkyl radical containing from three to seven
carbon atoms and includes cyclopropyl, cyclobutyl,
cyclopentyl, cyclohexyl and cycloheptyl.

15

The term "C_{4-10} (alkylcycloalkyl) as used herein means
a cycloalkyl radical containing from three to seven
carbon atoms linked to an alkyl radical, the linked
radicals containing up to ten carbon atoms; for

5   example, cyclopropylmethyl, cyclopentylethyl,
cyclohexylmethyl, cyclohexylethyl or cycloheptyl-
ethyl.

The term "C_{2-10} alkenyl" as used herein, either alone

10   or in combination with another radical, means an
alkyl radical as defined above containing from 2 to
10 carbon atoms, and further containing at least one
double bond. For example alkenyl includes allyl.

15   The term "C_{3-4} alkylene" as used herein means a
divalent alkyl radical derived by the removal of two
hydrogen atoms from a straight or branched chain
aliphatic hydrocarbon containing from three to four
carbon atoms and includes, for example, $-CH_2CH_2CH_2-$,

20      $CH(CH_3)CH_2CH_2-$, $-CH_2C(CH_3)_2-$ and $- CH_2CH_2CH_2CH_2-$.

The term "C_{1-6} alkoxy" as used herein, either alone or
in combination with another radical, means the
radical $-O-C_{1-6}$ alkyl wherein alkyl is as defined

25   above containing up to six carbon atoms. Alkoxy
includes methoxy, ethoxy, propoxy, 1-methylethoxy,
butoxy and 1,1-dimethylethoxy. The latter radical is
known commonly as *tert*-butoxy.

30   The term "C_6 or C_{10} aryl" as used herein, either alone
or in combination with another radical, means either
an aromatic monocyclic system containing 6 carbon
atoms or an aromatic cyclic system containing 10

carbon atoms.   For example, aryl includes phenyl or
naphthalene.

The term "C$_{7-16}$ aralkyl" as used herein, either alone
5   or in combination with another radical, means an aryl
as defined above linked through an alkyl group,
wherein alkyl is as defined above containing from 1
to 6 carbon atoms. Aralkyl includes for example
benzyl, and butylphenyl.
10

The term "carboxy(lower)alkyl" as used herein, either
alone or in combination with another radical, means a
carboxyl group (COOH) linked through a (lower)alkyl
group as defined above and includes for example
15   butyric acid or the groups:

or
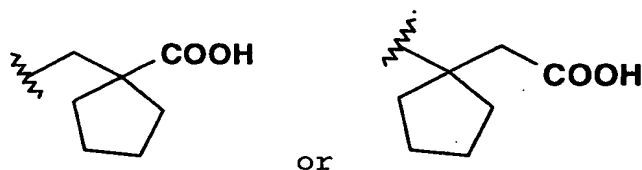
The term "cyclic" or "cyclic system" as used herein,
20   either alone or in combination with another radical,
means a monovalent radical derived by removal of a
hydrogen from a saturated or unsaturated cyclic
hydrocarbon, containing from three to seven carbon
atoms, unless otherwise indicated and optioonally
25   conctaing one or more heteroatom. The term cyclic or
cyclic system includes, for example, cyclopropane,
cyclopentane, cyclohexane, cyclohexene, decalin,
tetralin, indene, and naphthalene.

30   The term "heterocycle" as used herein, either alone
or in combination with another radical, means a

monovalent radical derived by removal of a hydrogen
from a five-, six-, or seven-membered saturated or
unsaturated heterocycle containing from one to four
heteroatoms selected from nitrogen, oxygen and

5   sulfur. Examples of suitable heterocycles include:
pyrrolidine, tetrahydrofuran, thiazolidine, pyrrole,
thiophene, diazepine, 1H-imidazole, 1-methyl-1H-
imidazole, isoxazole, thiazole, 2-methylthiazole, 2-
aminothiazole, piperidine, 1,4-dioxane, 4-morpholine,

10   pyridine, 2-methylpyridine, pyrimidine, 4-
methylpyrimidine and 2,4-dimethylpyrimidine.


The term "heterocyclic system" as used herein, either
alone or in combination with another radical, means a

15   heterocycle as defined above fused to one or more
other cycle be it a heretocycle or any other cycle.
Examples of suitable heterocyclic systems include:
thiazolo[4,5-b]-pyridine, quinoline, or indole.

20   The term "pharmaceutically acceptable ester" as used
herein, either alone or in combination with another
radical, means esters of the compound of formula I in
which any of the carboxyl functions of the molecule,
but preferably the carboxy terminus, is replaced by

25   an alkoxycarbonyl function:

$$\underset{\textstyle OR}{\overset{\textstyle O}{\|}}$$
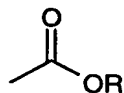
in which the **R** moiety of the ester is selected from
alkyl (e.g. methyl, ethyl, *n*-propyl, t-butyl, *n*-
butyl); alkoxyalkyl (e.g. methoxymethyl); alkoxyacyl

30   (e.g. acetoxymethyl); aralkyl (e.g. benzyl);
aryloxyalkyl (e.g. phenoxymethyl); aryl (e.g.
phenyl), optionally substituted with halogen, C_{1-4}
alkyl or C_{1-4} alkoxy. Other suitable prodrug esters

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can be found in Design of prodrugs, Bundgaard, H. Ed.
Elsevier (1985) incorporated herewith by reference.
Such pharmaceutically acceptable esters are usually
hydrolyzed *in vivo* when injected in a mammal and
5   transformed into the acid form of the compound of
formula I.

The term "pharmaceutically acceptable salt" as used
herein includes those derived from pharmaceutically
10   acceptable bases. Examples of suitable bases include
choline, ethanolamine and ethylenediamine. Na^+, K^+,
and Ca^{++} salts are also contemplated to be within the
scope of the invention (also see Pharmaceutical
salts, Birge, S.M. et al., J. Pharm. Sci. (1977), 66,
15   1-19, incorporated herein by reference).

Preferred embodiments
A further preferred group of compounds are
20   represented by formula Ia:

P6   P5   P4   P3   P2   P1

(Ia)

wherein **Y** is H or C_{1-6} alkyl;

a is 0 or 1;

b is 0 or 1;

25   **B** is an acyl derivative of formula R_{11}-C(O)-wherein
R_{11} is (i) C_{1-10} alkyl optionally substituted with
carboxyl, C_{1-6} alkanoyloxy or C_{1-6} alkoxy; (ii) C_{3-7}
cycloalkyl optionally substituted with carboxyl, (C_{1-6}
alkoxy)carbonyl or phenylmethoxycarbonyl; (iii) C_{3-7}

cycloalkyl substituted with carboxyl and one to three
C_{1-6} alkyl substituents (iv) C_{4-10} (alkylcycloalkyl)
optionally substituted on the cycloalkyl portion with
carboxy, (C_{1-6} alkoxy)carbonyl or
phenylmethyoxycarbonyl; (v)

$$HOOC\text{-}(C_{1-6}alkyl)\text{-}N \diagup \diagdown NCOO\text{-}(aryl\ or\ C_{1-6}\ alkylaryl)\ ,$$

$$HO\diagdown \underset{O}{\overset{}{C}}\diagdown (CH_2)_{1-5} \quad or \quad HO\diagdown \underset{O}{\overset{}{C}}\diagdown (CH_2)_{1-5}\ ;$$

(v) C_6 or C_{10} aryl or C_{7-16} aralkyl optionally
substituted with C_{1-6} alkyl;

R_6, when present, is C_{1-6} alkyl substituted with
carboxyl;

R_5, when present, is C_{1-6} alkyl optionally substituted
with carboxyl; and

R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}
(alkylcycloalkyl);

R_3, W, R_1, R_1' and A are as defined above.

Preferably, B is an acyl derivative of formula
$R_{11}C(O)$- wherein R_{11} is: C_{1-6} alkyl optionally
substituted with carboxyl, C_{1-6} alkanoyloxy or C_{1-6}
alkoxy;

C_{3-7} cycloalkyl optionally substituted with carboxyl,
MeOC(O), EtOC(O) or BnOC(O);

3-carboxypropionyl (DAD) or 4-carboxybutyryl (DAE);
or

$$HOOCCH_2N \diagup \diagdown NCOOBn$$

.
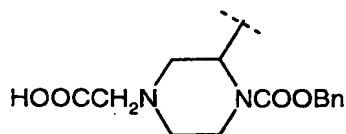
More preferably, **B** is acetyl, 3-carboxypropionyl, 4-carboxylbutyryl, AcOCH$_2$C(O), Me$_3$COC(O),
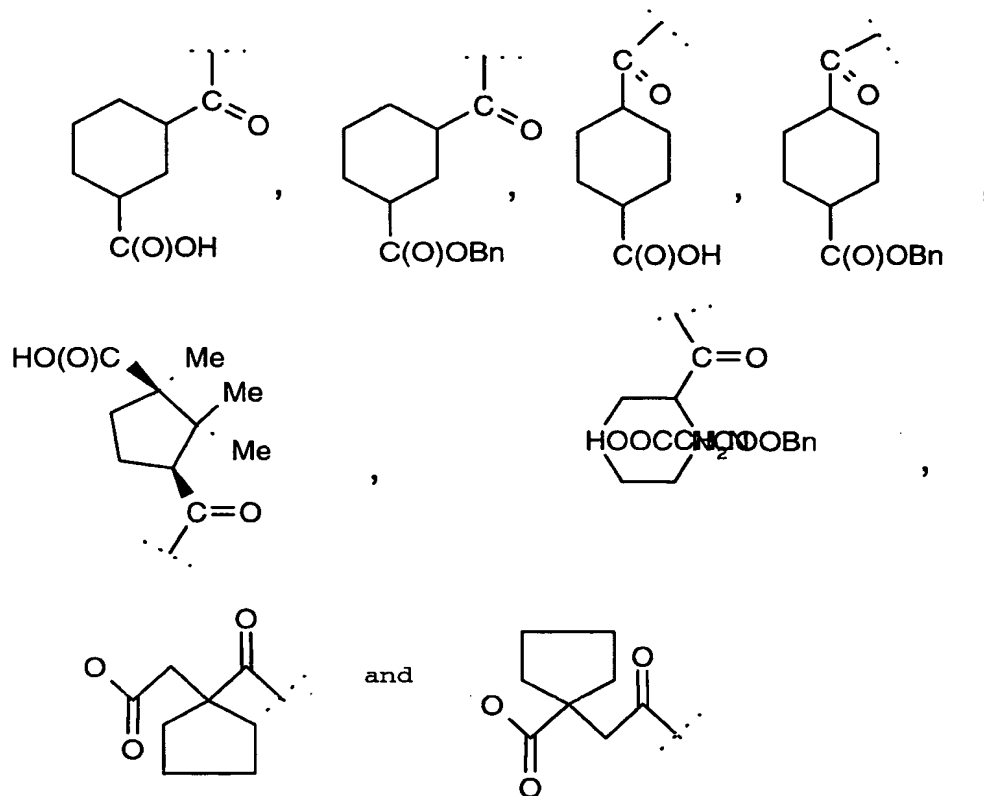
Still, more preferably, **B** is acetyl, 3-carboxypropionyl (DAD), 4-carboxybutyryl (DAE), AcOCH$_2$C(O),
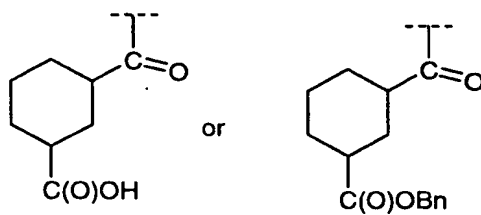
Most preferably, **B** is acetyl.

Preferably, R_6, when present, is the side chain of
Asp or Glu.

Most preferably, R_6, when present, is the side chain
of Asp.

5   Alternatively, preferably, **a** is 0 and then R_6 is
absent.

Preferably, R_5, when present, is the side chain of an
amino acid selected from the group consisting of: D-

10 Asp, L-Asp, D-Glu, L-Glu, D-Val, L-Val, D-*tert*-
butylglycine (Tbg), and L-Tbg.

More preferably, R_5, when present, is the side chain
of D-Asp, D-Val, or D-Glu.

Most preferably, R_5, when present, is the side chain

15 of D-Glu.

Alternatively, preferably **a** is 0 and **b** is 0, and then
both R_6 and R_5 are absent.

Alternatively, another preferred group of compounds

20 are represented by formula (Ib):

P4   P3   P2   P1
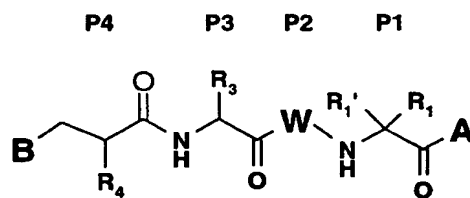
(Ib)

wherein **B** is preferably an amide of formula
$R_{11a}N(R_{11b})C(O)-$ wherein R_{11a} is preferably C_{1-6} alkyl,
C_{3-6} cycloalkyl, C_{3-7} (alkylcylcoalkyl) optionally

25 substituted with carboxy, C_{1-3} carboxyalkyl, C_6 aryl,
C_{7-10} arylalkyl, 2-tetrahydrofuranylmethyl, or 2-
thiazolidylmethyl;

and R_{11b} is preferably C_{1-4} alkyl substituted with
carboxyl.

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Most preferably, R_{11a} is cyclopropylmethyl, isopropyl, carboxyethyl, benzylmethyl, benzyl, or 2-tetrahydrofuranylmethyl. More preferably R_{11b} is C_{1-4} alkyl substituted with carboxyl. Most preferably, R_{11b} is ethyl carboxyl.

Compounds of the invention include compounds of formula I wherein, preferably, R_4 is selected from the group consisting of: isopropyl, cyclohexyl, tert-butyl, 1-methylpropyl, and 2-methylpropyl.
More preferably, R_4 is cyclohexyl or 1-methylpropyl.
Most preferably, R_4 is cyclohexyl.

Compounds of the invention include compounds of formula I wherein Z is preferably oxo.

Compounds of the invention include compounds of formula I wherein preferably, R_3 is the side chain of an amino acid selected from the group consisting of: Ile, allo-Ile, Chg, Cha, Val, Tbg or Glu.
More preferably, R_3 is the side chain of Val, Tbg or Chg.
Most preferably, R_3 is the side chain of Val.

Compounds of the invention include compounds of formula I wherein preferably, W is a group of formula II:
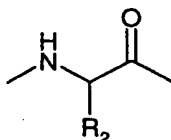
wherein R_2 is C_{1-8} alkyl; C_{1-8} alkyl substituted with carboxyl, C_{1-6} alkoxycarbonyl, benzyloxycarbonyl or

benzylaminocarbonyl; C_{3-7} cycloalkyl or benzyl.
Preferably, R_2 is the side of chain of Abu, Leu, Phe,
Cha, Val, Ala, Asp, Glu, Glu(Obn), or Glu(NHBn).
Most preferably, R_2 is the side chain of Asp,

5   aminobutyric acid (Abu) or Val.

Still, more preferably, compounds of the invention
include compounds of formula I wherein W is a group
of formula II':
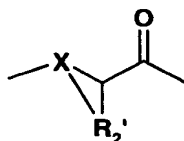
10

wherein preferably, X is CH or N.

More preferably R_2' is a C_3 or C_4 alkylene (shown in

15   bold) that joins X to form a 5- or 6-membered ring of
formula III:
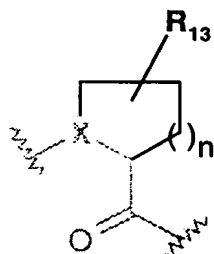
Formula III

R_2' being optionally substituted at any position with

20   R_{13}, wherein X is CH or N; n is 1 or 2, and R_{13} is as
defined below.

Most preferably, X is N. For example, preferably R_2'
is propyl joined to X wherein X is nitrogen to form a

25   proline substituted with R_{13} at P2.

Most preferably R_2' is the side chain of proline

substituted at the 3-, 4-, or 5-position with R_{13},

wherein R_{13} is as defined below.

5   Still, most preferably R_2' is the side chain of

proline (as shown in bold) substituted with R_{13} at

the 4-position with the stereochemistry shown in

formula III':
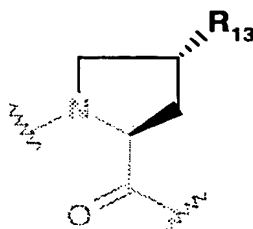
Formula III'

10   wherein R_{13} is preferably OH; SH; NH_2; carboxyl; R_{12};

OR_{12}, SR_{12}, NHR_{12} or $NR_{12}R_{12}'$ wherein R_{12} and R_{12}' are

independently:

        cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or

        cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl,

15   said alkyl or alkenyl optionally substituted

        with NH_2, OH, SH, halo, or carboxyl; said alkyl

        or alkenyl optionally containing at least one

        heteroatom independently selected from the group

        consisting of: O, S, and N; or

20   R_{12} and R_{12}' are independently C_6 or C_{10} aryl or

        C_{7-16} aralkyl optionally substituted with C_{1-6}

        alkyl, NH_2, OH, SH, halo, carboxyl or

        carboxy(lower)alkyl; said aryl or aralkyl

        optionally containing at least one heteroatom

25   independently selected from the group consisting

        of: O, S, and N;

        said cyclic alkyl, cyclic alkenyl, aryl or

        aralkyl being optionally fused with a second 5-,

        6-, or 7-membered ring to form a cyclic system

25

or heterocyclic system, said second ring being
optionally substituted with NH_2, OH, SH, halo,
carboxyl or carboxy(lower)alkyl; said second
ring optionally containing at least one

5   heteroatom independently selected from the group
consisting of: O, S, and N.

More preferably, R_{13} is OR_{12} or SR_{12} wherein R_{12} is a C_6
or C_{10} aryl or C_{7-16} aralkyl, said first aryl or

10   aralkyl optionally substituted with C_{1-6} alkyl, C_{3-7}
cycloalkyl, NH_2, OH, SH, halo, C_{1-6} alkoxy, carboxyl,
carboxy(lower)alkyl, or a second aryl or aralkyl;
said first and second aryl or aralkyl optionally
containing at least one heteroatom selected

15   independently from the group consisting of: O, S, and
N.

Most preferably, R_{13} is Bn; $PhCH_2CH_2$; $PhCH_2CH_2CH_2$; O-
Bn; o-tolylmethoxy; m-tolylmethoxy; p-tolylmethoxy;

20   1-naphtyloxy; 2-naphtyloxy; 1-naphthalenylmethoxy; 2-
naphthalenylmethoxy; (4-tert-butyl)methoxy; (3I-
Ph)CH_2O; (4Br-Ph)O; (2Br-Ph)O; (3Br-Ph)O; (4I-Ph)O;
(3Br-Ph)CH_2O; (3,5-Br_2-Ph)CH_2O;

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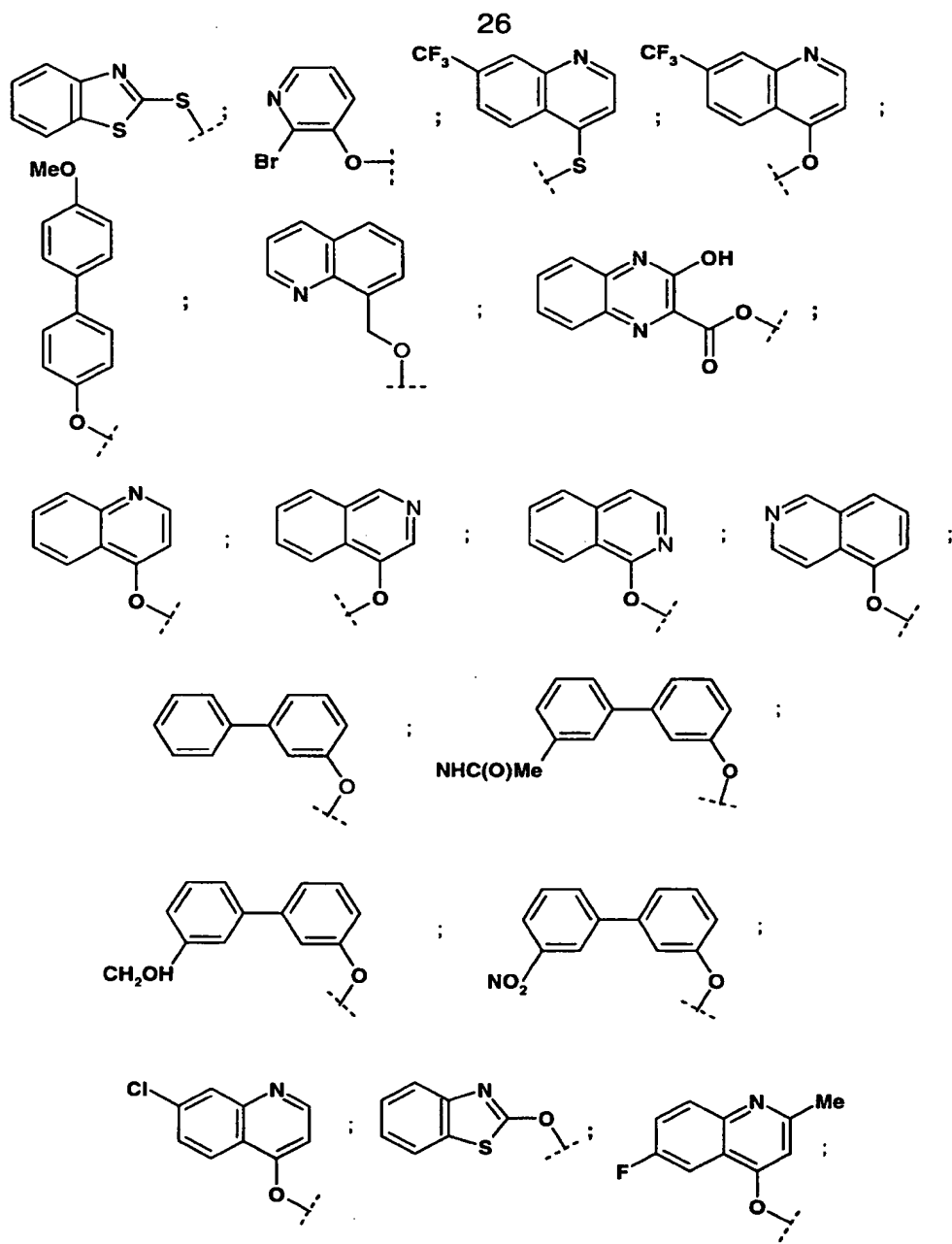
Still most preferably, **R₁₃** is PhCH₂CH₂CH₂; O-Bn; 1-
naphtyloxy; 2-naphtyloxy; 1-naphthalenylmethoxy; 2-
naphthalenylmethoxy;

27
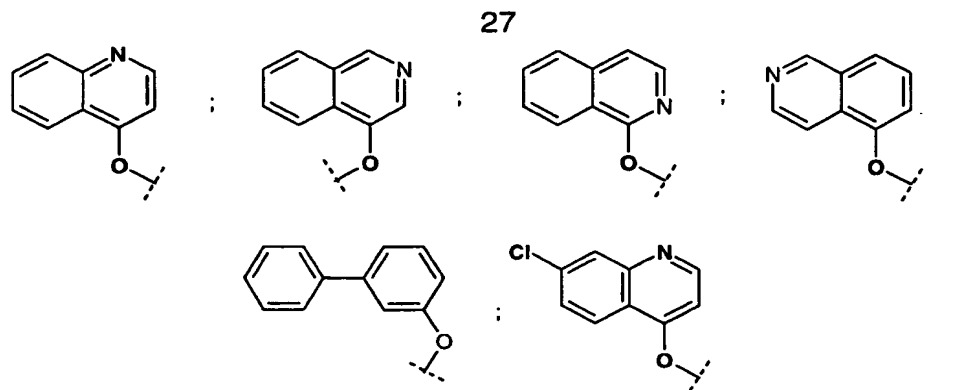
Further include within the invention are compounds of
formula I wherein **R₁'** is preferably hydrogen and **R₁**
is C₁₋₆ alkyl optionally substituted with thiol. For
example, **R₁** is preferably the side chain of the amino
acid selected from the group consisting of: cysteine
(Cys), aminobutyric acid (Abu), norvaline (Nva), or
allylglycine (AlGly).
More preferably, **R₁'** is H and **R₁** is propyl. For
example, **R₁** is more preferably the side chain of the
amino acid Nva.

Alternatively, preferably, **R₁'** and **R₁** together form a
3- to 6-membered ring, said ring being optionally
substituted with ethyl. For example, **R₁'** and **R₁**
together form preferably a cyclopropyl, a cyclobutyl,
a cyclopentyl, or a cyclohexyl ring. Alternatively,
more preferably, **R₁'** and **R₁** together form a
cyclopropyl, For example, **R₁'** and **R₁** together can be
the side chain (shown in bold) of the following amino
acid:
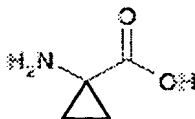
referred to as 1-aminocyclopropylcarboxylic acid
(Acca).

Further included in the present invention are
compounds of fomrula I wherein **A** is preferably
hydroxy, a salt or an ester thereof. More preferably,
5   **A** is hydroxy or an ester thereof. Most preferably, **A**
is hydroxy.

More preferably, the ester is C_{1-6} alkoxy, or (aryl
C_{1-6}-alkoxy). Most preferably, the ester is methoxy,
10 ethoxy, phenoxy, or benzyloxy

Included in the scope of the invention are compounds
of formula I wherein **Q** is CH_2, **a** is 0, **b** is 0, and
then **B** is an amide of formula $R_{11a}N(R_{11b})$-C(O)- wherein
15 **R$_{11a}$** is C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{3-7}
(alkylcylcoalkyl) optionally substituted with
carboxy, C_{1-3} carboxyalkyl, phenyl, C_{7-10} arylalkyl,
2-tetrahydrofuranylmethyl, or 2-thiazolidylmethyl;
and **R$_{11b}$** is phenyl; or C_{1-6} alkyl substituted with
20 carboxyl or C_{1-4} carboxyalkyl;
or
Q is N-**Y** wherein **Y** is H or C_{1-6} alkyl; **a** is 0 or 1; **b**
is 0 or 1; and **B** is an acyl derivative of formula
R_{11}-C(O)- wherein **R$_{11}$** is (i) C_{1-6} alkyl, C_{1-6} alkyl
25 substituted with carboxyl, MeC(O)O-, MeO-, EtO-,
$MeCH_2CH_2O$- or Me_3C-O-; (ii) cyclopentyl or cyclohexyl
optionally substituted with carboxyl; (iv) C_{4-10}
(alkylcycloalkyl) optionally substituted on the
cycloalkyl portion with carboxyl;
30 (v)
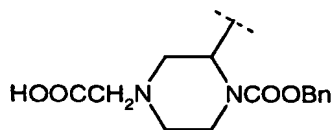
(vi) phenyl, benzyl or phenylethyl;

R_6, when present, is CH_2COOH or CH_2CH_2COOH,

R_5, when present, is C_{1-6} alkyl or CH_2COOH or

CH_2CH_2COOH;

and when Q is either CH_2 or $N-Y$,

R_4 is C_{1-6} alkyl, C_{3-7} cycloalkyl or C_{4-10}

(alkylcycloalkyl);

Z is oxo or thio;

R_3 is C_{1-6} alkyl; C_{3-7} cycloalkyl or C_{4-10}

(alkylcycloalkyl);

W is a group of formula II wherein R_2 is C_{1-10} alkyl,

C_{3-10} cycloalkyl, C_{7-11} aralkyl; CH_2COOH or CH_2CH_2COOH;

or W is a group of formula II' wherein X is N or CH

and $R_{2'}$ is the divalent radical $-CH_2CH_2CH_2-$ or $-$

$CH_2CH_2CH_2CH_2-$ which together with X and the carbon

atom to which X and $R_{2'}$ are attached form a 5- or 6-

membered ring, said ring optionally substituted with

OR_{12}, $C(O)OR_{12}$, SR_{12}, NHR_{12} or $NR_{12}R_{12'}$ wherein R_{12} and

$R_{12'}$ are independently:

    cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or

    cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl,

    said alkyl or alkenyl optionally substituted

    with NH_2, OH, SH, halo, or carboxyl; said alkyl

    or alkenyl optionally containing at least one

    heteroatom independently selected from the grop

    consisting of: O, S, and N; or R_{12} and $R_{12'}$ are

    independently C_6 or C_{10} aryl or C_{7-16} aralkyl

    optionally substituted with C_{1-6} alkyl, CF_3, NH_2,

    OH, SH, halo, carboxyl, C_{1-6} alkyl substituted

    with carboxyl, or phenyl optionally substituted

    with C_{1-6} alkyl, C_{1-6} alkoxy or halo; said aryl or

    aralkyl optionally containing at least one

heteroatom independently selected from the group
consisting of: O, S, and N; said cyclic alkyl,
cyclic alkenyl, aryl or aralkyl being optionally
fused with a second 5-, 6-, or 7-membered ring
5   to form a cyclic system or heterocyclic system,
said second ring being optionally substituted
with NH_2, OH, SH, halo, carboxyl or C_{1-6} alkyl
substituted with carboxyl; said second ring
optionally containing at least one heteroatom
10   independently selected from the group consisting
of: O, S, and N; or **X** is N; and $R_{2'}$ is –
$CH_2CH_2CH_2$– or $-CH_2CH_2CH_2CH-$ which together with **X**
and the carbon atom to which **X** and $R_{2'}$ are
attached form a 5- or 6-membered ring, which in
15   turn is fused to a phenyl to form a cyclic
system wherein the phenyl ring is substituted
with OR_{12} wherein R_{12} is phenylmethyl or
phenylethyl;

$R_{1'}$ is hydrogen and R_1 is methyl, thiomethyl, 1-
20 methylethyl, propyl, 1-methylpropyl, 2-
(methylthio)ethyl or 2-propylene; or $R_{1'}$ and R_1
together with the carbon atom to which they are
attached form a cyclopropyl which may optionally be
substituted with ethyl; and

25 **A** is hydroxy or a pharmaceutically acceptable salt
thereof; C_{1-6} alkoxy, or (aryl C_{1-6}-alkoxy).

Included in the scope of the invention are compounds
of formula Ia, wherein **B** is an acyl derivative of
30 formula $R_{11}-C(O)-$ wherein R_{11} is C_{1-6} alkoxy, C_{1-10}
alkyl optionally substituted with carboxyl; C_{3-7}
cycloalkyl optionally substituted with carboxyl or
benzylcarboxy; or
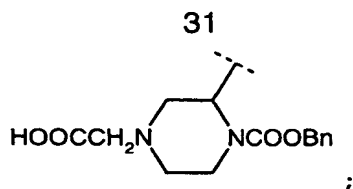
31

$$HOOCCH_2N \quad NCOOBn$$

;

R$_6$ is absent;

R$_5$ is absent;

R$_4$ is C$_{1-10}$ alkyl, C$_{3-7}$ cycloalkyl or C$_{4-10}$
(alkylcycloalkyl);

R$_3$ is C$_{1-10}$ alkyl, C$_{3-7}$ cycloalkyl or C$_{4-10}$
(alkylcycloalkyl);
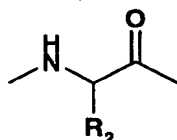
W is a group of formula II:

$$\overset{O}{\underset{R_2}{N}}$$ Formula II

wherein **R$_2$** is C$_{1-6}$ alkyl; C$_{3-6}$ cycloalkyl; C$_{1-6}$ alkyl
substituted with carboxyl; C$_6$ or C$_{10}$ aryl; or C$_{7-11}$
aralkyl;

or

W is a group of formula II':

$$\overset{O}{\underset{R_2'}{X}}$$ Formula II'

wherein **X** is N; and **R$_2$.** is as defined in claim 1,
and

A is hydroxy or a pharmaceutically acceptable salt
thereof; methoxy, ethoxy, phenoxy, or benzyloxy.

Included in the scope of the invention are compounds
of formula Ia, wherein **B** is acetyl, 3-
carboxypropionyl, 4-carboxylbutyryl, AcOCH$_2$C(O),
Me$_3$COC(O),
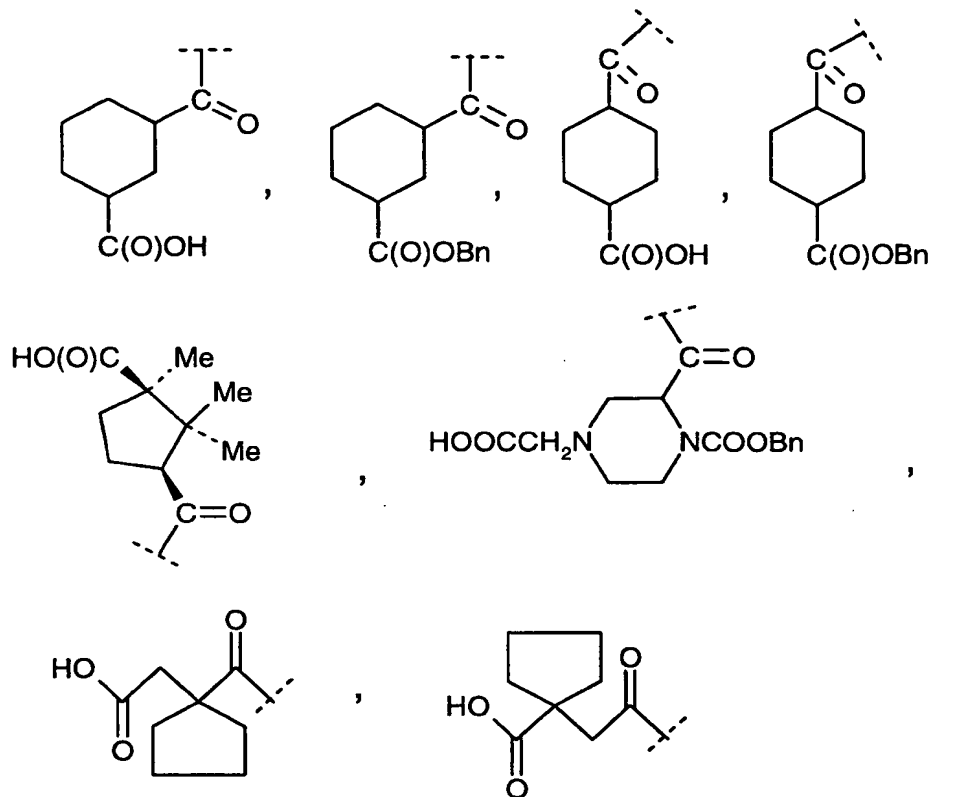
Y is H or Me, a is 0 or 1, b is 0 or 1,

R_6, when present, is the side chain of Asp or Glu,

R_5, when present, is the side chain of Asp, D-Asp,

Glu, D-Glu, Val, D-Val or Tbg,

R_4 is the side chain of Val, Chg, Tbg, Ile or Leu,

Z is oxo or thioxo,

R_3 is hydrogen or the side chain of Ile, Chg, Val, Glu;

W is Abu, Leu, Phe, Val, Ala, Glu, Glu(OBn); or

W is group of formula III':

(III')
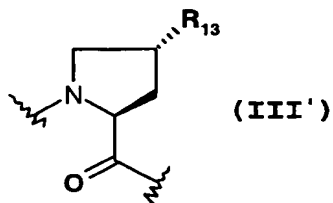
wherein R_{13} is Bn, PhCH₂CH₂, PhCH₂CH₂CH₂, O-Bn, o-
tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-
naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-*tert*-
butyl)benzyloxy, (3I-Ph)CH₂O, (4Br-Ph)O, (2Br-Ph)O,

5   (3Br-Ph)O, (4I-Ph)O, (3Br-Ph)CH₂O, (3,5-Br₂-Ph)CH₂O,
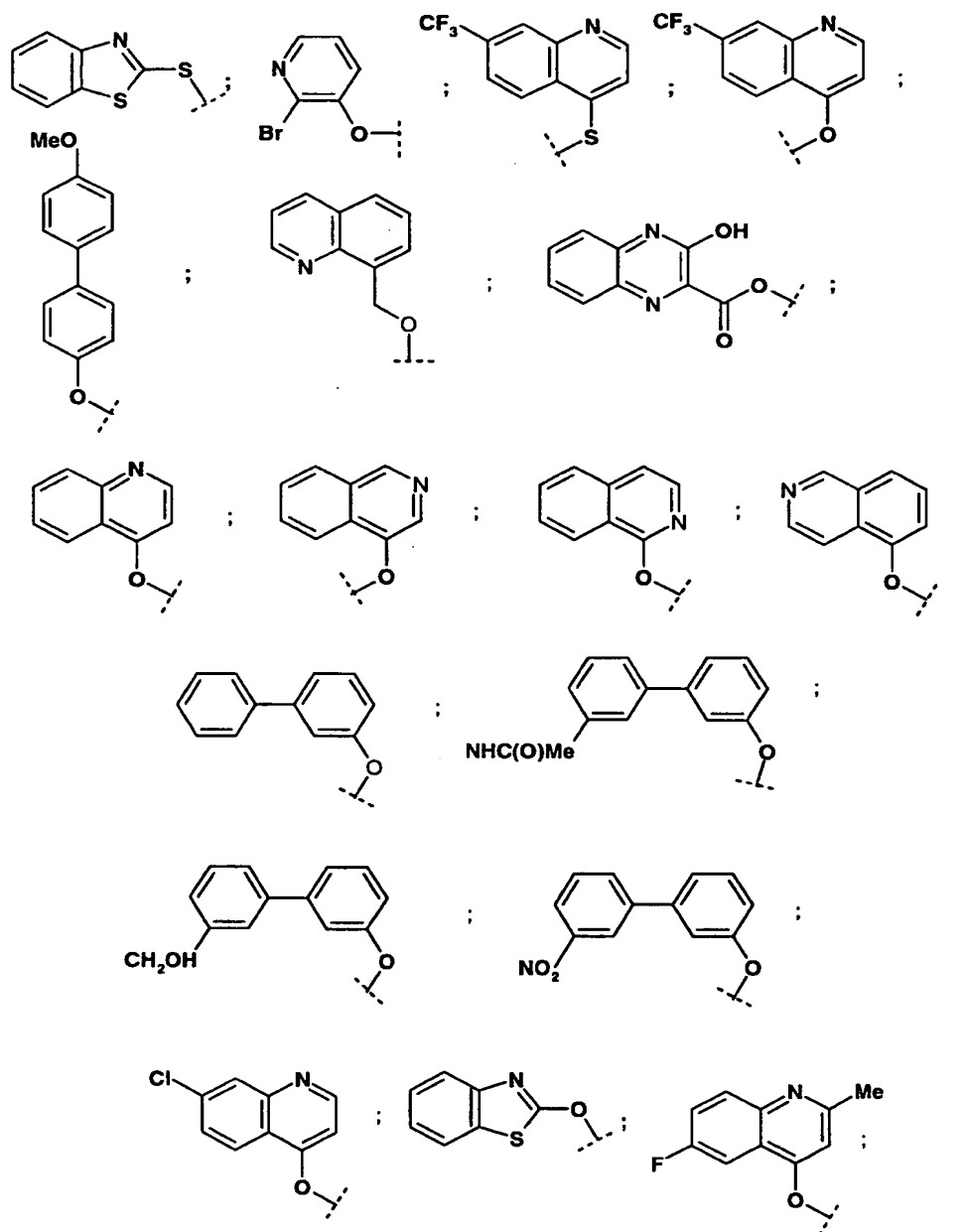
34

$R_{1'}$ is H and R_1 is the side chain of Cys, Abu, Nva or allylglycine; or

$R_{1'}$ and R_1 together with the carbon atom to which they are attached form a cyclopropyl; and **A** is hydroxyl.

Also included in the scope of the invention are compounds of formula Ib, wherein **B** is an amide of formula $R_{11a}N(R_{11b})-C(O)-$ wherein R_{11a} is C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{3-7} (alkylcylcoalkyl) optionally substituted with carboxy, C_{1-3} carboxyalkyl, phenyl, C_{7-10} arylalkyl, 2-tetrahydrofuranylmethyl, or 2-thiazolidylmethyl; and R_{11b} is phenyl; or C_{1-6} alkyl substituted with carboxyl or C_{1-4} carboxyalkyl;

R_4 is cyclohexyl;

Z is oxo;

R_3 is hydrogen or the side chain of Ile, Chg, Val, Glu;

W is Abu, Leu, Phe, Val, Ala, Glu, Glu(OBn); or

W is group of formula III':

(III')
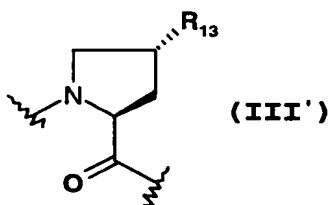
wherein R_{13} is Bn, $PhCH_2CH_2$, $PhCH_2CH_2CH_2$, O-Bn, o-tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-*tert*-butyl)methoxy, $(3I-Ph)CH_2O$, $(4Br-Ph)O$, $(2Br-Ph)O$, $(3Br-Ph)O$, $(4I-Ph)O$, $(3Br-Ph)CH_2O$, $(3,5-Br_2-Ph)CH_2O$,

35

R₁′ is H and R₁ is the side chain of Cys, Abu, Nva or allylglycine; or

5   R₁′ and R₁ together with the carbon atom to which they are attached form a cyclopropyl; and A is hydroxyl.

Also included within the scope of the present invention are compounds of formula I:

wherein **B** is an acyl derivative of formula $R_{11}-C(O)-$
wherein R_{11} is C_{1-10} alkyl optionally substituted with
carboxyl; C_{3-7} cycloalkyl optionally substituted with
carboxyl; or a C_{4-10} (alkylcycloalkyl) optionally

5   substituted on the cycloalkyl portion with carboxyl;
or R_{11} is C_6 or C_{10} aryl or C_{7-16} aralkyl optionally
substituted with a C_{1-6} alkyl
a is 0 or 1;
R_6, when present, is C_{1-6} alkyl optionally substituted

10   with carboxyl;
b is 0 or 1;
R_5, when present, is C_{1-6} alkyl optionally substituted
with carboxyl;
Q is **N-Y** wherein **Y** is H or C_{1-6} alkyl;

15   R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}
(alkylcycloalkyl);
Z is oxo;
R_3 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}
(alkylcycloalkyl);

20   **W** is a group of formula II:
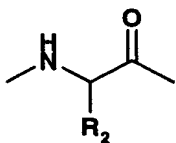
$$\underset{R_2}{\overset{\displaystyle \quad\quad O}{\underset{\displaystyle H}{N}}}$$ Formula II

wherein R_2 is C_{1-6} alkyl; C_{1-6} alkyl optionally

25   substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-16}
aralkyl;

W is a group of formula II':

37
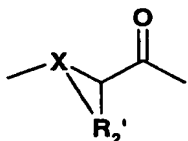
Formula II'

wherein **X** is CH or N; and

R₂' is C₃₋₄ alkyl that joins **X** to form a 5- or 6-

5   membered ring, said ring optionally substituted with

OH; SH; NH₂; carboxyl; **R₁₂**; OR₁₂, SR₁₂, NHR₁₂ or NR₁₂R₁₂'

wherein **R₁₂** and **R₁₂'** are independently:

cyclic C₃₋₁₆ alkyl or acyclic C₁₋₁₆ alkyl or

cyclic C₃₋₁₆ alkenyl or acyclic C₂₋₁₆ alkenyl,

10   said alkyl or alkenyl optionally substituted

with NH₂, OH, SH, halo, or carboxyl; said alkyl

or alkenyl optionally containing at least one

heteroatom selected independently from the group

consisting of: O, S, and N; or

15   **R₁₂** and **R₁₂'** are independently C₆ or C₁₀ aryl or

C₇₋₁₆ aralkyl optionally substituted with C₁₋₆

alkyl, NH₂, OH, SH, halo, carboxyl or C₁₋₆ alkyl

substituted with carboxyl; said aryl or aralkyl

optionally containing at least one heteroatom

20   selected independently from the group consisting

of: O, S, and N;

said cyclic alkyl, cyclic alkenyl, aryl or

aralkyl being optionally fused with a second 5-,

6-, or 7-membered ring to form a cyclic system

25   or heterocyclic system, said second ring being

optionally substituted with NH₂, OH, SH, halo,

carboxyl or carboxy(lower)alkyl; said second

ring optionally containing at least one

heteroatom selected independently from the group

30   consisting of: O, S, and N;

and

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R_1', is hydrogen, and R_1 is C_{1-6} alkyl optionally substituted with thiol, or C_{2-6} alkenyl; or R_1' and R_1 together form a 3- to 6-membered ring optionally substituted with C_{1-6} alkyl; and

5   A is OH or a pharmaceutically acceptable salt or ester thereof.

Finally, included in the scope of the invention are all compounds of formula I presented in Tables 1 to

10   4.

According to an alternate embodiment, the pharmaceutical compositions of this invention may additionally comprise an antiviral agent. Examples

15   of antiviral agents include, ribavirin and amantadine.

According to another alternate embodiment, the pharmaceutical compositions of this invention may

20   additionally comprise other inhibitors of HCV protease.

According to yet another alternate embodiment, the pharmaceutical compositions of this invention may

25   additionally comprise an inhibitor of other targets in the HCV life cycle, such as helicase, polymerase, or metalloprotease.

The pharmaceutical compositions of this invention may

30   be administered orally, parenterally or via an implanted reservoir. We prefer oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable

carriers, adjuvants or vehicles. In some cases, the
pH of the formulation may be adjusted with
pharmaceutically acceptable acids, bases or buffers
to enhance the stability of the formulated compound
5   or its delivery form. The term parenteral as used
herein includes subcutaneous, intracutaneous,
intravenous, intramuscular, intra-articular,
intrasynovial, intrasternal, intrathecal, and
intralesional injection or infusion techniques.
10   The pharmaceutical compositions may be in the form of
a sterile injectable preparation, for example, as a
sterile injectable aqueous or oleaginous suspension.
This suspension may be formulated according to
techniques known in the art using suitable dispersing
15   or wetting agents (such as, for example. Tween 80)
and suspending agents.

The pharmaceutical compositions of this invention may
be orally administered in any orally acceptable
20   dosage form including, but not limited to, capsules,
tablets, and aqueous suspensions and solutions. In
the case of tablets for oral use, carriers which are
commonly used include lactose and corn starch.
Lubricating agents, such as magnesium stearate, are
25   also typically added. For oral administration in a
capsule form, useful diluents include lactose and
dried corn starch. When aqueous suspensions are
administered orally, the active ingredient is
combined with emulsifying and suspending agents. If
30   desired, certain sweetening and/or flavoring and/or
coloring agents may be added.

Other suitable vehicles or carriers for the above
noted formulations and compositions can be found in

40

standard pharmaceutical texts, e.g. in "Remington's
Pharmaceutical Sciences", The Science and Practice of
Pharmacy, 19th Ed. Mack Publishing Company, Easton,
Penn., (1995).

5

Dosage levels of between about 0.01 and about 100
mg/kg body weight per day, preferably between about
0.5 and about 75 mg/kg body weight per day of the
protease inhibitor compounds described herein are
10   useful in a monotherapy for the prevention and
treatment of HCV mediated disease. Typically, the
pharmaceutical compositions of this invention will be
administered from about 1 to about 5 times per day or
alternatively, as a continuous infusion. Such
15   administration can be used as a chronic or acute
therapy. The amount of active ingredient that may be
combined with the carrier materials to produce a
single dosage form will vary depending upon the host
treated and the particular mode of administration. A
20   typical preparation will contain from about 5% to
about 95% active compound (w/w). Preferably, such
preparations contain from about 20% to about 80%
active compound.

25   As the skilled artisan will appreciate, lower or
higher doses than those recited above may be
required. Specific dosage and treatment regimens for
any particular patient will depend upon a variety of
factors, including the activity of the specific
30   compound employed, the age, body weight, general
health status, sex, diet, time of administration,
rate of excretion, drug combination, the severity and
course of the infection, the patient's disposition to
the infection and the judgment of the treating

physician. Generally, treatment is initiated with
small dosages substantially less than the optimum
dose of the peptide. Thereafter, the dosage is
increased by small increments until the optimum

5   effect under the circumstances is reached. In
general, the compound is most desirably administered
at a concentration level that will generally afford
antivirally effective results without causing any
harmful or deleterious side effects.

10

When the compositions of this invention comprise a
combination of a compound of formula I and one or
more additional therapeutic or prophylactic agent,
both the compound and the additional agent should be

15   present at dosage levels of between about 10 to 100%,
and more preferably between about 10 and 80% of the
dosage normally administered in a monotherapy
regimen.

20   When these compounds or their pharmaceutically
acceptable salts are formulated together with a
pharmaceutically acceptable carrier, the resulting
composition may be administered in vivo to mammals,
such as man, to inhibit HCV NS3 protease or to treat

25   or prevent HCV virus infection. Such treatment may
also be achieved using the compounds of this
invention in combination with agents which include,
but are not limited to: immunomodulatory agents, such
as α-, β-, or γ-interferons; other antiviral agents

30   such as ribavirin, amantadine; other inhibitors of
HCV NS3 protease; inhibitors of other targets in the
HCV life cycle such as helicase, polymerase,
metalloprotease, or internal ribosome entry; or
combinations thereof. The additional agents may be

combined with the compounds of this invention to
create a single dosage form. Alternatively these
additional agents may be separately administered to a
mammal as part of a multiple dosage form.

5

Accordingly, another embodiment of this invention
provides methods of inhibiting HVC NS3 protease
activity in mammals by administering a compound of
the formula I, wherein the substituents are as
10   defined above.

In a preferred embodiment, these methods are useful
in decreasing HCV NS3 protease activity in a mammal.
If the pharmaceutical composition comprises only a
15   compound of this invention as the active component,
such methods may additionally comprise the step of
administering to said mammal an agent selected from
an immunomodulatory agent, an antiviral agent, a HCV
protease inhibitor, or an inhibitor of other targets
20   in the HCV life cycle such as helicase, polymerase,
or metallo protease. Such additional agent may be
administered to the mammal prior to, concurrently
with, or following the administration of the
compositions of this invention.

25

In an alternate preferred embodiment, these methods
are useful for inhibiting viral replication in a
mammal. Such methods are useful in treating or
preventing HCV disease. If the pharmaceutical
30   composition comprises only a compound of this
invention as the active component, such methods may
additionally comprise the step of administering to
said mammal an agent selected from an
immunomodulatory agent, an antiviral agent, a HCV

protease inhibitor, or an inhibitor of other targets
in the HCV life cycle. Such additional agent may be
administered to the mammal prior to, concurrently
with, or following the administration of the
5   composition according to this invention.

The compounds set forth herein may also be used as
laboratory reagents. The compounds of this invention
may also be used to treat or prevent viral
10   contamination of materials and therefore reduce the
risk of viral infection of laboratory or medical
personnel or patients who come in contact with such
materials (e.g. blood, tissue, surgical instruments
and garments, laboratory instruments and garments,
15   and blood collection apparatuses and materials).

PROCESS
The compounds of the present invention were
synthesized according to the process as illustrated
20   in scheme I (wherein PG1 is a carboxyl protecting
group and PG2 is an amino protecting group):

Scheme I

P1-PG1   +   PG2-P2 $\xrightarrow{\text{a}}$ PG2-P2-P1-PG1

$\xleftarrow{\text{b}}$

P2-P1-PG1 + PG2-P3 $\xrightarrow{\text{c}}$ PG2-P3-P2-P1-PG1

$\xleftarrow{\text{d}}$

P3-P2-P1-PG1 + PG2-P4 $\xrightarrow{\text{e}}$ PG2-P4-P3-P2-P1-PG1

$\xleftarrow{\text{f}}$

P4-P3-P2-P1-PG1 + PG2-P5-OH $\xrightarrow{\text{g}}$ PG2-P5-P4-P3-P2-P1-PG1

$\xleftarrow{\text{h}}$

P5-P4-P3-P2-P1-PG1 + PG2-P6 $\xrightarrow{\text{i}}$ PG2-P6-P5-P4-P3-P2-P1-PG1

$\xleftarrow{\text{j}}$

P6-P5-P4-P3-P2-P1-PG1 + BOH $\xrightarrow{\text{k}}$ B-P6-P5-P4-P3-P2-P1-PG1

$\xleftarrow{\text{l}}$

B-P6-P5-P4-P3-P2-P1-OH

(I)
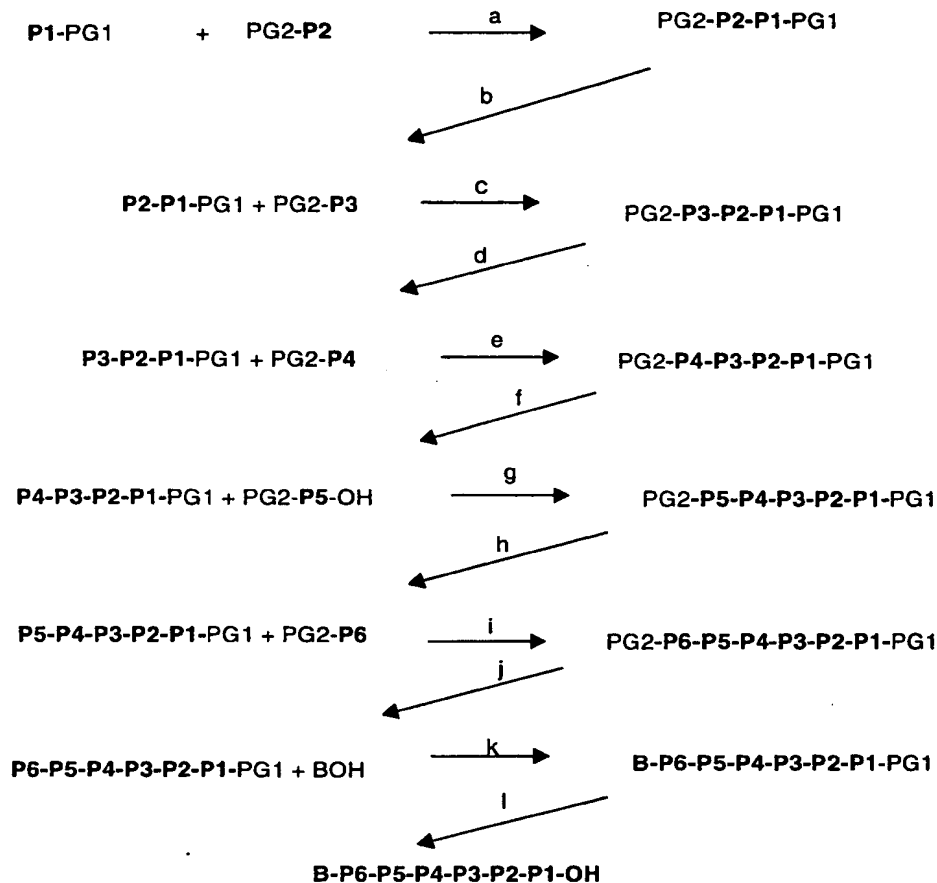
Briefly, the P1, P2, P3, P4, and optionally P5 and P6
can be linked by well known peptide coupling
techniques. The P1, P2, P3, P4, and P5 and P6 groups
5   may be linked together in any order as long as the
final compound corresponds to peptides of formula **I**.
For example, P6 can be linked to P5 to give P5-P6
that is linked to P4-P3-P2-P1 ; or P6 linked to P5-
P4-P3-P2 then linked to an appropriately C-terminal
10   protected P1.

Generally, peptides are elongated by deprotecting the
α-amino group of the N-terminal residue and coupling

the unprotected carboxyl group of the next suitably
N-protected amino acid through a peptide linkage
using the methods described. This deprotection and
coupling procedure is repeated until the desired
5   sequence is obtained. This coupling can be performed
with the constituent amino acids in stepwise fashion,
as depicted in Scheme I, or by condensation of
fragments (two or several amino acids), or
combination of both processes, or by solid phase
10   peptide synthesis according to the method originally
described in Merrifield, J. Am. Chem. Soc. (1963),
85, 2149-2154, the disclosure of which is hereby
incorporated by reference.

15   Coupling between two amino acids, an amino acid and a
peptide, or two peptide fragments can be carried out
using standard coupling procedures such as the azide
method, mixed carbonic-carboxylic acid anhydride
(isobutyl chloroformate) method, carbodiimide
20   (dicyclohexylcarbodiimide, diisopropylcarbodiimide,
or water-soluble carbodiimide) method, active ester
(p-nitrophenyl ester, N-hydroxysuccinic imido ester)
method, Woodward reagent K-method,
carbonyldiimidazole method, phosphorus reagents or
25   oxidation-reduction methods. Some of these methods
(especially the carbodiimide method) can be enhanced
by adding 1-hydroxybenzotriazole. These coupling
reactions can be performed in either solution (liquid
phase) or solid phase.
30
More explicitly, the coupling step involves the
dehydrative coupling of a free carboxyl of one
reactant with the free amino group of the other
reactant in the presence of a coupling agent to form

a linking amide bond. Descriptions of such coupling
agents are found in general textbooks on peptide
chemistry, for example, M. Bodanszky, "Peptide
Chemistry", 2nd rev ed., Springer-Verlag, Berlin,
5   Germany, (1993). Examples of suitable coupling
agents are N,N'-dicyclohexylcarbodiimide, 1-
hydroxybenzotriazole in the presence of N,N'-
dicyclohexylcarbodiimide or N-ethyl-N'-[(3-
dimethylamino)propyl]carbodiimide. A very practical
10   and useful coupling agent is the commercially
available (benzotriazol-1-yloxy)tris-
(dimethylamino)phosphonium hexafluorophosphate,
either by itself or in the presence of 1-
hydroxybenzotriazole. Another very practical and
15   useful coupling agent is commercially available 2-
(1H-benzotriazol-1-yl)-N, N, N', N'-
tetramethyluronium tetrafluoroborate. Still another
very practical and useful coupling agent is
commercially available O-(7-azabenzotriazol-1-yl)-
20   N,N,N',N'-tetramethyluronium hexafluorophosphate.

The coupling reaction is conducted in an inert
solvent, e.g. dichloromethane, acetonitrile or
dimethylformamide. An excess of a tertiary amine,
25   e.g. diisopropylethylamine, N-methylmorpholine or N-
methylpyrrolidine, is added to maintain the reaction
mixture at a pH of about 8. The reaction temperature
usually ranges between 0°C and 50°C and the reaction
time usually ranges between 15 min and 24 h.
30

When a solid phase synthetic approach is employed,
the C-terminal carboxylic acid is attached to an
insoluble carrier (usually polystyrene). These
insoluble carriers contain a group that will react

with the carboxylic group to form a bond that is
stable to the elongation conditions but readily
cleaved later. Examples of which are: chloro- or
bromomethyl resin, hydroxymethyl resin, and

5   aminomethyl resin. Many of these resins are
commercially available with the desired C-terminal
amino acid already incorporated. Alternatively, the
amino acid can be incorporated on the solid support
by known methods Wang, S.-S., J. Am. Chem. Soc.,

10   (1973), 95, 1328; Atherton, E.; Shepard, R.C. "Solid-
phase peptide synthesis; a practical approach" IRL
Press: Oxford, (1989); 131-148. In addition to the
foregoing, other methods of peptide synthesis are
described in Stewart and Young, "Solid Phase Peptide

15   Synthesis", 2nd ed., Pierce Chemical Co., Rockford,
IL (1984); Gross, Meienhofer, Udenfriend, Eds., "The
Peptides: Analysis, Synthesis, Biology", Vol. 1, 2,
3, 5, and 9, Academic Press, New-York, (1980-1987);
Bodansky et al., "The Practice of Peptide Synthesis"

20   Springer-Verlag, New-York (1984), the disclosures of
which are hereby incorporated by reference.

The functional groups of the constituent amino acids
generally must be protected during the coupling

25   reactions to avoid formation of undesired bonds. The
protecting groups that can be used are listed in
Greene, "Protective Groups in Organic Chemistry",
John Wiley & Sons, New York (1981) and "The Peptides:
Analysis, Synthesis, Biology", Vol. 3, Academic

30   Press, New York (1981), the disclosures of which are
hereby incorporated by reference.

The α-carboxyl group of the C-terminal residue is
usually protected as an ester (PG1) that can be

cleaved to give the carboxylic acid. Protecting
groups that can be used include: 1) alkyl esters such
as methyl, trimethylsilylethyl and t-butyl, 2)
aralkyl esters such as benzyl and substituted benzyl,

5   or 3) esters that can be cleaved by mild base
treatment or mild reductive means such as
trichloroethyl and phenacyl esters.

The α-amino group of each amino acid to be coupled to

10   the growing peptide chain must be protected (PG2).
Any protecting group known in the art can be used.
Examples of such groups include: 1) acyl groups such
as formyl, trifluoroacetyl, phthalyl, and p-
toluenesulfonyl; 2) aromatic carbamate groups such as

15   benzyloxycarbonyl (Cbz or Z) and substituted
benzyloxycarbonyls, and 9-fluorenylmethyloxycarbonyl
(Fmoc); 3) aliphatic carbamate groups such as tert-
butyloxycarbonyl (Boc), ethoxycarbonyl,
diisopropylmethoxycarbonyl, and allyloxycarbonyl; 4)

20   cyclic alkyl carbamate groups such as
cyclopentyloxycarbonyl and adamantyloxycarbonyl; 5)
alkyl groups such as triphenylmethyl and benzyl; 6)
trialkylsilyl such as trimethylsilyl; and 7) thiol
containing groups such as phenylthiocarbonyl and

25   dithiasuccinoyl. The preferred α-amino protecting
group is either Boc or Fmoc. Many amino acid
derivatives suitably protected for peptide synthesis
are commercially available.

30   The α-amino protecting group of the newly added amino
acid residue is cleaved prior to the coupling of the
next amino acid. When the Boc group is used, the
methods of choice are trifluoroacetic acid, neat or
in dichloromethane, or HCl in dioxane or in ethyl

acetate. The resulting ammonium salt is then
neutralized either prior to the coupling or *in situ*
with basic solutions such as aqueous buffers, or
tertiary amines in dichloromethane or acetonitrile or

5   dimethylformamide. When the Fmoc group is used, the
reagents of choice are piperidine or substituted
piperidine in dimethylformamide, but any secondary
amine can be used. The deprotection is carried out
at a temperature between 0°C and room temperature

10   (RT), usually 20-22°C.

Any of the amino acids having side chain
functionalities must be protected during the
preparation of the peptide using any of the above-

15   described groups. Those skilled in the art will
appreciate that the selection and use of appropriate
protecting groups for these side chain
functionalities depend upon the amino acid and
presence of other protecting groups in the peptide.

20   The selection of such protecting groups is important
in that the group must not be removed during the
deprotection and coupling of the α-amino group.

For example, when Boc is used as the α-amino

25   protecting group, the following side chain protecting
groups are suitable: *p*-toluenesulfonyl (tosyl)
moieties can be used to protect the amino side chain
of amino acids such as Lys and Arg; acetamidomethyl,
benzyl (Bn), or *t*-butylsulfonyl moieties can be used

30   to protect the sulfide containing side chain of
cysteine; benzyl (Bn) ethers can be used to protect
the hydroxy containing side chains of serine,
threonine or hydroxyproline; and benzyl esters can be

50

used to protect the carboxy containing side chains of
aspartic acid and glutamic acid.

When Fmoc is chosen for the α-amine protection,
5   usually *tert*-butyl based protecting groups are
    acceptable. For instance, Boc can be used for lysine
    and arginine, *tert*-butyl ether for serine, threonine
    and hydroxyproline, and *tert*-butyl ester for aspartic
    acid and glutamic acid. Triphenylmethyl (Trityl)
10 moiety can be used to protect the sulfide containing
    side chain of cysteine.

    Once the elongation of the peptide is completed all
    of the protecting groups are removed. When a liquid
15 phase synthesis is used, the protecting groups are
    removed in whatever manner is dictated by the choice
    of protecting groups. These procedures are well
    known to those skilled in the art.

20 When a solid phase synthesis is used, the peptide is
    cleaved from the resin simultaneously with the
    removal of the protecting groups. When the Boc
    protection method is used in the synthesis, treatment
    with anhydrous HF containing additives such as
25 dimethyl sulfide, anisole, thioanisole, or *p*-cresol
    at 0°C is the preferred method for cleaving the
    peptide from the resin. The cleavage of the peptide
    can also be accomplished by other acid reagents such
    as trifluoromethanesulfonic acid/ trifluoroacetic
30 acid mixtures. If the Fmoc protection method is
    used, the N-terminal Fmoc group is cleaved with
    reagents described earlier. The other protecting
    groups and the peptide are cleaved from the resin

using solution of trifluoroacetic acid and various
additives such as anisole, etc.

When **Q** is CH_2, **a** is 0, **b** is 0 and **B** is $R_{11a}N(R_{11b})C(O)$,
5   the compounds were prepared according to a method
analogous to the general method described for the
peptides in Scheme I using a readily available
succinyl intermediate, t-BuO-C(O)CH_2CH(**R$_4$**)-CO-PG1
(e.g. PG1= 2-oxo-4-substituted-oxazolidin-3-yl).
10   This succinyl intermediate can easily be prepared
according to the method of Evans'et al (J. Am. Chem.
Soc. (1982), <u>104</u>, 1737) using the appropriate 4-
substituted-3-acyl-2-oxazolidinone in the presence of
a strong base such as lithium diisopropylamide or
15   sodium bis(trimethylsilyl)amide and t-butyl
bromoacetate. After cleavage of the 2-oxazolidinone
moiety with LiOOH (Evans'et al., Tetrahedron Lett.
(1987), <u>28</u>, 6141), the resulting acid was coupled to
the P3-P2-P1-PG1 segment to give t-BuO-C(O)-
20   CH_2CH(**R$_4$**)-CO-P3-P2-P1-PG1. The latter was treated
with hydrogen chloride to selectively convert the
terminal t-butyl ester into the corresponding acid
that was finally coupled to $R_{11a}NH(R_{11b})$ to give, after
removal of the protective group(s), the desired
25   peptide derivative. The amines $R_{11a}NH(R_{11b})$ are
commercially available or the synthesis is well known
in the art. A specific embodiment of this process is
presented in Example 18.

30   Alternatively, starting with the same succinyl
intermediate (t-BuO-C(O)CH_2CH(**R$_4$**)-CO-PG1), the
sequence of reactions can be inverted to introduce
first $R_{11a}NH(R_{11b})$ and then P3-P2-P1-PG1 to give the
desired peptide derivative.

52

Synthesis of capping group B and P6, P5, P4, and P3 moieties

Different capping groups **B** are introduced to
protected P6, P5, P4, the whole peptide or to any
peptide segment with an appropriate acyl chloride
that is either available commercially or for which
the synthesis is well known in the art.

Different **P6** to **P3** moieties are available
commercially or the synthesis is well known in the
art.

Synthesis of P2 moieties.

1. Synthesis of precursors:
A) Synthesis of haloarylmethane derivatives.

The preparation of halomethyl-8-quinoline **IId**
was done according to the procedure of K.N.
Campbell et al., J. Amer. Chem. Soc., (1946),
<u>68</u>, 1844.

Scheme II
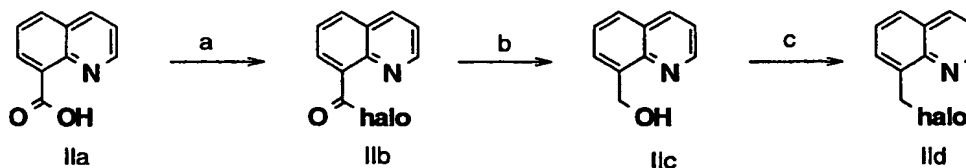
Briefly, 8-quinoline carboxylic acid **IIa** was
converted to the corresponding alcohol **IIc** by
reduction of the corresponding acyl halide **IIb**
with a reducing agent such as lithium aluminium
hydride. Treatment of alcohol **IIb** with the
appropriate hydrohaloacid gives the desired halo
derivative **IId**. A specific embodiments of this
process is presented in Example 1.

53

2. Synthesis of P2:

A) The synthesis of 4-substituted proline (wherein R^2 is attached to the ring via a carbon atom) (with the stereochemistry as shown):

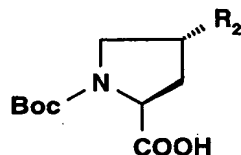is done as shown in Scheme III according to the procedures described by J. Ezquerra et al. (Tetrahedron, (1993), 38, 8665-8678) and C. Pedregal et al. (Tetrahedron Lett., (1994), 35, 2053-2056).

Scheme III
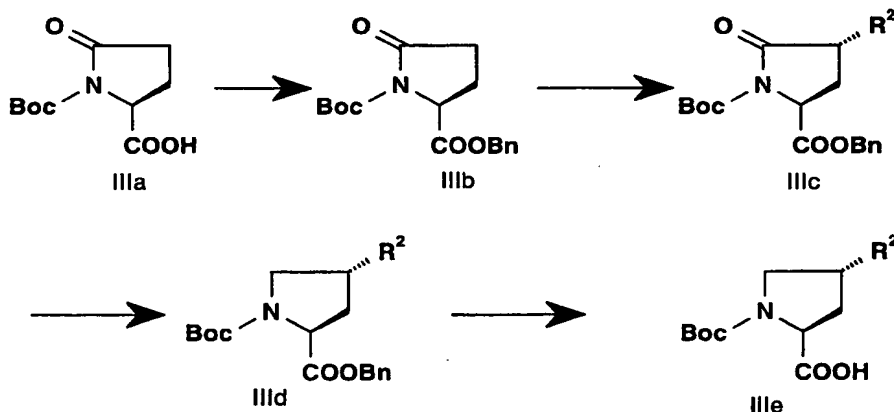
Briefly, Boc-pyroglutamic acid is protected as a benzyl ester. Treatment with a strong base such as lithium diisopropylamide followed by addition of an alkylating agent (Br-R^2 or I-R^2) gives the desired compounds **IIIe** after reduction of the amide and deprotection of the ester.

B) The synthesis of O-alkylated 4-(R)-hydroxyproline:

54

Boc-N pyrrolidine ring with O-R¹² substituent and COOH

may be carried out using the different processes
described below.

5   B.1)   When **R¹²** is aralkyl, the process can be
        carried out according to the procedure described
        by E.M. Smith et al. (J. Med. Chem. (1988), 31,
        875-885).   Briefly, commercially available Boc-
        4(R)-hydroxyproline is treated with a base such
10   as sodium hydride and the resulting alkoxide
        reacted with an alkylating agent (Br-**R¹²** or I-
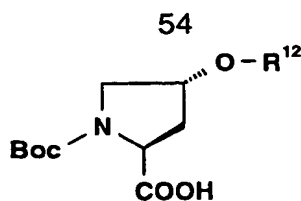   **R¹²**) to give the desired compounds.   Specific
        embodiments of this process are presented in
        Examples 3 and 4.

15

        B.2) When **R¹²** is aryl, the compounds can be
        prepared via a Mitsunobu reaction (Mitsunobu
        (1981), Synthesis, January, 1-28; Rano et al.,
        (1995), Tet. Lett. 36(22), 3779-3792; Krchnak et
20   al., (1995), Tet. Lett. 36(5), 62193-6196;
        Richter et al., (1994), Tet. Lett. 35(27), 4705-
        4706).   Briefly, commercially available Boc-
        4(S)-hydroxyproline methyl ester is treated with
        the appropriate aryl alcohol or thiol in the
25   presence of triphenylphosphine and
        diethylazodicarboxylate (DEAD) and the resulting
        ester is hydrolysed to the acid.   Specific
        embodiments of this process are presented in
        Examples 5 and 6.

55

Scheme IV

IVa   IVb
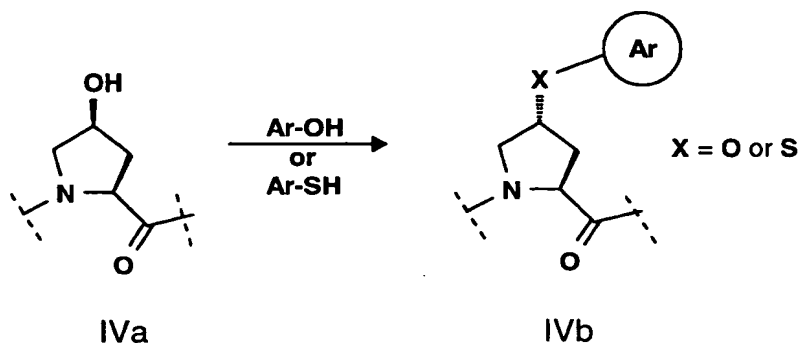
Alternatively, the Mitsunobu reaction can be produced
in solid phase (as shown in Scheme IV). The 96-well
block of the Model 396 synthesizer (advanced
ChemTech) is provided with aliquots of resin-bound
compound (**IVa**) and a variety of aryl alcohols or
thiols and appropriate reagents are added. After
incubation, each resin-bound product (**IVb**) is washed,
dried, and cleaved from the resin.

B.2.a)   A Suzuki reaction (Miyaura *et al.*,
(1981), Synth. Comm. 11, 513; Sato *et al.*,
(1989), Chem. Lett., 1405; Watanabe *et al.*,
(1992), Synlett., 207; Takayuki *et al.*,
(1993), J. Org. Chem. 58, 2201; Frenette *et
al.*, (1994), Tet. Lett. 35(49), 9177-9180;
Guiles *et al.*, (1996), J. Org. Chem. 61,
5169-5171) can also be used to further
functionalize the aryl substituent.

Examples

The present invention is illustrated in further
detail by the following non-limiting examples.

Temperatures are given in degrees Celsius. Solution
percentages express a weight to volume relationship,
and solution ratios express a volume to volume
relationship, unless stated otherwise. Nuclear
magnetic resonance (NMR) spectra were recorded on a
Bruker 400 MHz spectrometer; the chemical shifts (δ)
are reported in parts per million. Flash
chromatography was carried out on silica gel (SiO_2)
according to Still's flash chromatography technique
(W.C. Still et al., J. Org. Chem. (1978), <u>43</u>, 2923).

Abbreviations used in the examples include Bn:
benzyl; Boc: *tert*-butyloxycarbonyl {$Me_3COC(O)$}; BSA:
bovine serum albumin; CHAPS: 3-[(3-cholamidopropyl)-
dimethylammonio]-1-propanesulfonate; DBU: 1,8-
diazabicyclo[5.4.0]undec-7-ene; CH_2Cl_2= DCM:
methylene chloride; DIPEA: diisopropylethylamine;
DMAP: dimethylaminopyridine; DCC: 1,3-
dicyclohexylcarbodiimide; DME: 1,2-dimethyoxyethane;
DMF: dimethylformamide; DMSO: dimethylsulfoxide; DTT:
dithiothreitol or threo-1,4-dimercapto-2,3-
butanediol; EDTA: ethylenediaminetetraacetic acid;
Et: ethyl; EtOH: ethanol; EtOAc: ethyl acetate; Et_2O:
diethyl ether; HPLC: high performance liquid
chromatography; MS: mass spectrometry (MALDI-TOF:
Matrix Assisted Laser Disorption Ionisation-Time of
Flight, FAB: Fast Atom Bombardment);LAH: lithium
aluminum hydride; Me: methyl; MeOH: methanol; MES:
(2-{*N*-morpholino}ethane-sulfonic acid); NaHMDS:

sodium bis(trimethylsilyl)amide; NMM: *N*-
methylmorpholine; NMP: *N*-methylpyrrolidine; Pr:
propyl; Succ: 4-hydroxy-1,4-dioxobutyl; PNA: 4-
nitrophenylamino or p-nitroanalide; TBAF: tetra-*n*-
butylammonium fluoride; TCEP: tris(2-carboxyethyl)
phosphine hydrochloride; TFA: trifluoroacetic acid;
THF: tetrahydrofuran; TIS: triisopropylsilane; TLC:
thin layer chromatography; TMSE: trimethylsilylethyl;
Tris/HCl: tris(hydroxymethyl)aminomethane
hydrochloride.

Example 1

Synthesis of bromomethyl-8-quinoline (1):

(1)
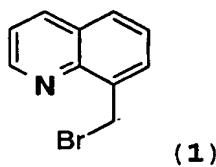
To commercially available 8-quinoline carboxylic acid
(2.5 g, 14.4 mmol) was added neat thionyl chloride
(10 ml, 144 mmol). This mixture was heated at 80°C
for 1 h before the excess thionyl chloride was
distilled off under reduced pressure. To the
resulting brownish solid was added absolute EtOH (15
mL) which was heated at 80°C for 1 h before being
concentrated *in vacuo*. The residue was partitioned
between EtOAc and saturated aqueous NaHCO₃, and the
organic phase dried (MgSO₄), filtered and
concentrated to give a brownish oil (2.8 g). This
material (ca. 14.4 mmol) was added dropwise over 35
min to a LAH (0.76 g, 20.2 mmol)/Et₂O suspension
which was cooled to -60°C. The reaction mixture was
slowly warmed to -35°C over 1.5 h before the reaction

was complete. The reaction was quenched with
$MgSO_4.10H_2O$ slowly over 30 min and then wet THF. The
mixture was partitioned between Et_2O and 10% aqueous
$NaHCO_3$.The organic phase was dried $(MgSO_4)$, filtered

5   and concentrated to give a yellowish solid (2.31 g,
80% over 2 steps) corresponding to the alcohol. The
alcohol (2.3 g, 11.44 mmol) was dissolved in AcOH/HBr
(20 mL, 30% solution from Aldrich) and heated at 70°C
for 2.5 h. The mixture was concentrated *in vacuo* to

10   dryness, partitioned between EtOAc (100 mL) and
saturated aqueous $NaHCO_3$ before being dried $(MgSO_4)$,
filtered and concentrated to give the desired
compound (**1**) as a brownish solid (2.54 g, 100%).

15

Example 2

Synthesis of Boc-4(R)-(3-phenylpropyl)proline (2d).

2a   2b

2c   2d
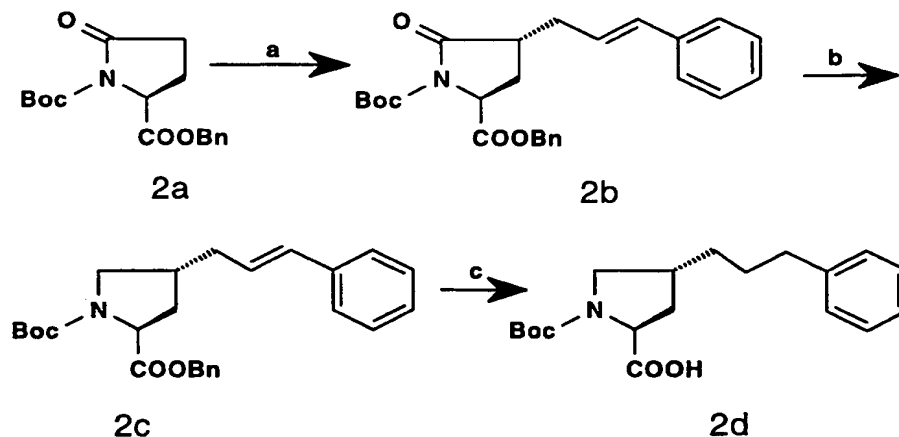
20   **a) Synthesis of compound 2b:**

To a solution of Boc-pyroglutamic acid benzyl ester
(**2a**) (prepared as described by A.L Johnson et al., J.
Med. Chem. (1985), 28, 1596-1602) (500 mg, 1.57 mmol)
in THF (10 mL) at -78 °C, was slowly added lithium

25   hexamethydisilylazide (1.72 mL, 1M solution in THF).

After stirring for 1 h at -78°C, cinnamyl bromide
(278 µL, 1.88 mmol) was added and the stirring
continued for an additional 2 h. The reaction
mixture was quenched with saturated ammonium chloride

5   solution and extracted with ethyl ether (3 x 20 mL).
The combined organic extracts were dried (MgSO₄),
filtered and concentrated. The residue was purified
by flash column chromatography (8:2 hexane:ethyl
acetate) to give compound **2b** as an off-white solid

10   (367 mg, 54% yield). ¹H NMR (CDCl₃): δ 7.35-7.19 (m,
10H), 6.43 (d, J=15 Hz, 1H), 6.11 (ddd, J=15, J'=J"=8
Hz, 1 H), 5.26 (d, J=16 Hz, 1H), 5.17 (d, J=16 Hz,
1H), 4.59 (dd, J=9.5, J'=2 Hz, 1 H), 2.83-2.70 (m,
2H), 2.41-2.34 (m, 1H), 2.22-2.16 (m, 1H), 2.10-2.02

15   (m, 1H) 1.42 (s, 9 H).

b) Synthesis of compound 2c:

At -78°C, lithium triethylborohydride (1M solution in
THF, 1.01 mL, 1.01 mmol) was added to a solution of

20   compound **2b** (367 mg, 0.843 mmol) in THF (5 mL), under
a nitrogen atmosphere. After 30 min, the reaction
mixture was quenched with saturated aqueous NaHCO₃ (2
mL) and warmed to 0°C. 30% H₂O₂ (5 drops) was added
and the mixture was stirred at 0°C for 20 min. The

25   organic volatiles were removed *in vacuo*, and the
aqueous layer was extracted with CH₂Cl₂ (3 x 10 mL).
The combined organic extracts were dried (MgSO₄),
filtered and concentrated. To a cold (-78°C)
solution of the residue and triethylsilane (134 µL,

30   0.843 mmol) in CH₂Cl₂ (3 mL) boron trifluoride
etherate (118 µL, 0.927 mmol) was added dropwise under
an atmosphere of nitrogen. After 30 min, additional
triethylsilane (134 µL) and boron trifluoride

etherate (118 μL) were added. After stirring for 2 h
at -78°C, the reaction mixture was quenched with
saturated aqueous NaHCO₃ (2 mL) and extracted with
DCM (3 x 10 mL). The combined organic extracts were
5   dried (MgSO₄), filtered and concentrated. The crude
product was purified by flash column chromatography
(8:2 hexane:ethyl acetate) to give compound **2c** as a
colorless oil (140 mg, 40% yield). ^{1}H NMR (CDCl₃)
indicated the presence of two rotamers: δ 7.34-7.22
10   (m, 10H), 6.38 (d, J=15.5 Hz, 1H), 6.15-6.08 (m, 1H),
5.29-5.07 (m, 2H), 4.44 (d, J=7 Hz, 1/3H), 4.33 (d,
J=7 Hz, 2/3H), 3.76 (dd, J=10.5, J'=8.5 Hz, 2/3H),
3.69 (dd, J=10.5, J'=8.5 Hz, 1/3H), 3.13 (dd, J=9,
J'=8.5 Hz, 2/3H), 3.05 (dd, J=9, J'=8.5 Hz, 1/3H),
15   2.47-2.40 (m, 1H), 2.35-2.22 (m, 2H) 2.15-1.85 (m,
2H), 1.45 (s, (3/9) 9H), 1.33 (s, (6/9) 9H).

c) Synthesis of compound 2d:

To a solution of compound **20** (140 mg, 0.332 mmol) in
20   ethanol (4 mL) was added 10% palladium on charcoal
(30 mg). The mixture was stirred under an atmosphere
of hydrogen for 2 h. The catalyst was removed by
passing the mixture through a Millipore: Millex - HV
0.45 μm filter. The clear solution was concentrated
25   to give the desired compound **2d** as a colorless oil
(115 mg, quant. yield). ^{1}H NMR (DMSO-d₆) indicated
the presence of two rotamers: δ 7.28-7.14 (m, 5H),
4.33 (br.s, 1H), 4.06-4.10, (m, 1H), 3.56-3.42 (m,
3H), 2.89-2.79 (m, 1H),), 2.53-2.49 (m, 1H, under
30   DMSO-d₆), 2.24-2.10 (m, 1H), 2.03-1.93 (m, 1H), 1.87-
1.75 (m, 1H), 1.62-1.45 (m, 2H), 1.38 (s, (3/9) 9H),
1.33 (s, (6/9) 9H).

Example 3

**Synthesis of Boc-4(R)-(naphthalen-1-ylmethoxy)
proline (3):**

(3)

5
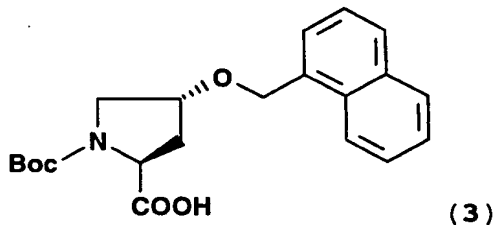
Commercially available Boc-4(R)-hydroxyproline (5.00
g, 21.6 mmol) was dissolved in THF (100 mL) and
cooled to 0°C. Sodium hydride (60% dispersion in oil,
10   1.85 g, 45.4 mmol) was added portionwise over 10
minutes and the suspension was stirred at RT for 1 h.
Then, 1-(bromomethyl)naphthalene (8.00 g, 36.2 mmol)
(prepared as described in E.A. Dixon et al. Can. J.
Chem., (1981), 59, 2629-2641) was added and the
15   mixture was heated at reflux for 18 h. The mixture
was poured into water (300 mL) and washed with
hexane. The aqueous layer was acidified with 10%
aqueous HCl and extracted twice with ethyl acetate.
The organic layers were combined and washed with
20   brine, dried (MgSO$_4$), filtered and concentrated. The
residue was purified by flash chromatography (49:49:2
hexane: ethyl acetate: acetic acid) to give the title
compound as a colorless oil (4.51 g, 56% yield). ^{1}H
NMR (DMSO-d$_6$) indicated the presence of two rotamers:
25   δ 8.05 (m, 1H), 7.94 (m, 1H), 7.29 (d, J=14 Hz, 1H),
7.55-7.45 (m, 4H), 4.96 (m, 2H), 4.26 (br. s, 1H),
4.12 (dd, J=J=8 Hz, 1H), 3.54-3.42 (m, 2H), 2.45-2.34
(m, 1H), 2.07-1.98 (m, 1H) 1.36 (s, (3/9) 9H), 1.34
(s, (6/9) 9H).

30

Example 4

**Synthesis of Boc-4(R)-(8-quinoline-methyloxy) proline
(4):**

(4)
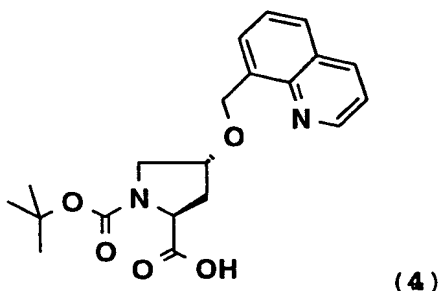
5   Boc-4(R)-hydroxyproline (1.96 g, 8.5 mmol) in
     anhydrous THF (20 mL) was added to a suspension of
     NaH (1.4 g, 60% in oil, 34 mmol) in THF (100 mL).
     This mixture was stirred 30 min before bromomethyl-8-
     quinoline from Example 1 (2.54 g, 11.44 mmol) was
10   added in THF (30 mL). The reaction mixture was heated
     at 70°C (5 h) before the excess NaH was destroyed
     carefully with wet THF. The reaction was concentrated
     in vacuo and the resulting material was dissolved in
     EtOAc and H_2O. The basic aqueous phase was separated
15   and acidified with 10% aqueous HCl to pH ~5 before
     being extracted with EtOAc (150 mL). The organic
     phase was dried ($MgSO_4$), filtered and concentrated to
     give a brown oil. Purification by flash
     chromatography (eluent: 10% $MeOH/CHCl_3$) gave the
20   desired compound as a pale yellow solid (2.73 g,
     86%). HPLC (97.5%); 1H-NMR (DMSO-d_6) shows rotamer
     populations in a 6:4 ratio, δ 12-11.4 (bs, 1H), 8.92
     (2 x d, J = 4.14 and 4.14 Hz, 1H), 8.38 (2 x d, J =
     8.27 and 8.27 Hz, 1H), 7.91 (d, J = 7.94 Hz, 1H),
25   7.77 (d, J = 7.0 Hz, 1H), 7.63-7.54 (m, 2H), 5.14 (2
     x s, 2H), 4.32-4.29 (m, 1H), 4.14-4.07 (m, 1H), 3.52-
     3.44 (m, 2H), 2.43-2.27 (m, 1H), 2.13-2.04 (m, 1H),
     1.36 and 1.34 (2 x s, 9H).

Example 5

Preparation of Boc-4*(R)*-(7-chloroquinoline-4-oxo)proline (5):

(5)
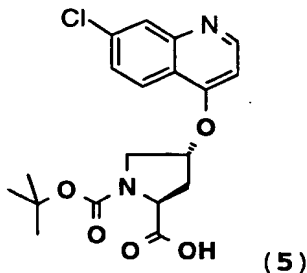
Commercially available Boc-4*(S)*-hydroxyproline methyl ester (500 mg, 2.04 mmol) and 7-chloro-4-hydroxyquinoline (440 mg, 2.45 mmol) were placed in dry THF (10 mL) at 0°C. Triphenylphosphine (641 mg, 2.95 mmol) was added, followed by slow addition of DIAD (426 mg, 2.45 mmol). The mixture was stirred at RT for 20 h. The reaction mixture was then concentrated, taken up in ethyl acetate and extracted three times with HCl 1N. The aqueous phase was basified with Na$_2$CO$_3$ and extracted twice with ethyl acetate. The organic layers were combined, dried over MgSO$_4$, filtered and concentrated to give a yellow oil. The oil was purified by flash chromatography to give compound (5) methyl ester as a white solid, 498 mg, 58% yield.

This methyl ester (400 mg, 0.986 mmol) was hydrolysed with 1M aqueous sodium hydroxide (1.7 mL, 1.7 mmol) in methanol (4 mL), at 0°C, for 3 h. The solution was concentrated to remove the methanol and neutralised with 1M aqueous HCl. The suspension was concentrated to dryness and taken up in methanol (20 mL), the salts were filtered off and the filtrate concentrated to give the desired compound (5) as   a

white solid, 387 mg, quant. yield.

^{1}H NMR (DMSO-d_6) (ca. 1:1 mixture of rotamers) δ 8.74
(d, J = 5 Hz, 1 H), 8.13-8.09 (m, 1 H), 7.99 and 7.98
(s, 1 H), 7.58 (d, J = 9 Hz, 1 H), 7.02 (d, J = 5 Hz,
5   1 H), 5.26-5.20 (m, 1 H), 4.10- 4.01 (m, 1 H), 3.81-
3.72 (m, 1 H), 3.59 (dd, J = 12, 10 Hz, 1 H), 2.41-
2.31 (m, 2 H), 1.34 and 1.31 (s, 9H).

Example 6

10   **General procedure for Mitsunobu reaction in solid
phase (Scheme IV)**

The polymer-bound peptide of general structure **IVa**
(0.327 mmoles of peptide per gram of Wang resin) was
dried under high vacuum in a desiccator over P_2O_5.
15   The 96-well block of the Advanced ChemTech Model 396
synthesizer was furnished with aliquots of **IVa** (120
mg, 0.04 mmol peptide per well) and each sample was
washed for 5 min with anhydrous CH_2Cl_2 (5x1200 μL)
and then with anhydrous THF (5x1500 μL). Anhydrous
20   THF (200 μL) was added to each sample and the
synthesizer was temporarily stopped to allow the
manual addition of reagents. Ph_3P (5 eq. in 400 μL
of anhydrous THF) and diethylazodicarboxylate (DIAD,
5 eq. in 250 μL of anhydrous THF)) were added to
25   each sample before the addition of a phenol or
thiophenol reagent (5 eq, 0.2 mmol, dissolved in 500
μL of anhydrous THF); a library of reagents was used
to produce the library of HCV protease inhibitors
described in this patent application. After the
30   addition of all reagents, the mixtures were shaken
for a total of 4 h with a 10 min delay after each
hour. Each resin-bound product was washed with THF
(2x1500 μL), DMF (4x1500 μL), isopropanol (4x1500

μL), CH$_2$Cl$_2$ (4x1500 μL) and finally methanol (2x1500

μL). The sample was dried under vacuum and then

treated with 40% TFA in CH$_2$Cl$_2$ for 1 h in order to

cleave the peptide product (general structure **IVb**)

5   from the resin. All products were purified by

preparative HPLC on a reversed phase C18 column using

a linear solvent gradient from 5% aqueous CH$_3$CN to

100% CH$_3$CN.

10   The following description is an example of the

further elaboration of the side chain R$_{12}$ at P2 by

the application of a biaryl synthesis via Suzuki

coupling on a solid support (cf. R. Frenette and R.W.

Friesen, Tetrahedron Lett. (1994), 35, 9177).

15

The precursor, aromatic bromide compound **238** of Table

2, was first synthesized from the polymer-bound

tetrapeptide having a *cis*-hydroxyproline at the P2

position and 4-bromophenol using the Mitsunobu

20   protocol described above.

Example 7

Suzuki Library of Reactions in Solid Phase Synthesis

25   All reactions were carried out in 16x100 mm, high

pressure screw-cap test tubes with teflon caps,

equipped with small magnetic stirring bars. For each

reaction, a degassed suspension of the polymer-bound

peptide (100 mg of Wang resin with 0.033 mmol of

30   bound peptide) was first added to the test tube,

followed by the addition of DME (2 mL), Pd(Ph$_3$P)$_3$ (~3

mg, 0.05 eq.), Na$_2$CO$_3$ (70 μL of a 2M solution in H$_2$O,

2.5 eq.) and one of the phenyl boronic acid reagents

from our library. The test tubes were flashed with

nitrogen gas, sealed and placed in an oil bath at
80°C. All of the reactions were stirred gently and
allowed to proceed for 15-18 h. Each resin bound
peptide product was subsequently transferred into a

5   plastic filtration tube, washed with DME:H_2O (1:1, 5x
2 mL), DME (5x 2 mL), methanol (5x 2 mL), CH_3CN (5x 2
mL), CH_2Cl_2 (5x 2 mL) and dried under high vacuum.
Each product was cleaved from the resin by treating
the sample with 45% TFA in CH_2Cl_2 (1 mL) for 1 hour.

10   All products were purified by preparative HPLC on a
reversed phase C18 column using a solvent linear
gradient from 5% aqueous CH_3CN to 100% CH_3CN.

Example 8

15   **Preparation of a library of Ac-Chg-Val-Hyp(aryl)-
Acca-OH**

This compound was synthesized in accordance with the
protocol of Example 6 where appropriate peptides were

20   used.

Example 9

Synthesis of Polymer-Bound Compound #246 of Table 2.

25
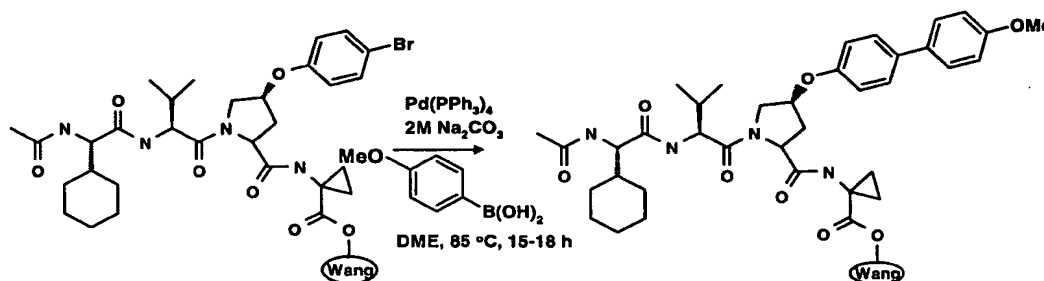
The synthesis of compound **246** was done according to
the process Example 7.

Compound 246:

ES⁻ MS m/z 675.3 [(M-H)⁻]; ~95% pure by C18 reversed
phase HPLC; Mixture of two rotamers in a ratio of
~1:3 based on ^{1}H NMR

5   ^{1}H NMR of major rotamer (400 MHz, DMSO): δ 8.44 (s,
1H), 7.84 (d, J=8.6 Hz, 1H), 7.82 (d, J=~8.6 Hz, 1H),
7.54 (bd, J=8.3 Hz, 4H), 6.99 (d, J=8.9 Hz, 2H), 6.98
(d, J=8.9 Hz, 2H), 5.11 (bs, 1H), 4.29-4.34 (m, 2H),
4.21 (bt, J=7.8 Hz, 1H), 3.94-4.02 (m, 2H), 3.78 (s,
10   3H), 2.29-2.33 (m, 2H), 2.15-2.21 (m, 1H), 1.95-1.99
(m, 1H), 1.83 (s, 3H), 1.45-1.70 (m, 8H), 1.33-1.40
(m, 1H), 1.20-1.28 (m, 1H), 1.02-1.18 (m, 2H), ~0.9-
1.02 (m, 2H), 0.90 (d, J= 6.7 Hz, 3H) 0.84 (d, J=6.7
Hz, 3H).

15

Example 10
General procedure for coupling reactions done in
solution {See also R. Knorr et al., Tetrahedron
**Letters, 30, 1927 (1989).}**

20

The reactants, i.e. a free amine (1 eq.) (or its
hydrochloride salt) and the free carboxylic acid (1
eq.) were dissolved in CH₂Cl₂, CH₃CN or DMF. Under a
nitrogen atmosphere, four equivalents of *N*-
25   methylmorpholine and 1.05 equivalents of the coupling
agent were added to the stirred solution. After 20
min, one equivalent of the second reactant, i.e. a
free carboxylic acid was added. (Practical and
efficient coupling reagents for this purpose are
30   (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium
hexafluorophosphate (HOBT) or preferably 2-(1H-
benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium
tetrafluoroborate (TBTU) or O-(7-azabenzotriazol-1-
yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate

(HATU). The reaction was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc. The solution was washed

5   successively with 10% aqueous citric acid, saturated aqueous NaHCO$_3$ and brine. The organic phase was dried (MgSO$_4$), filtered and concentrated under reduced pressure. When the residue was purified, it was done by flash chromatography as defined above.

10

Example 11

Synthesis of "tripeptide segment": Ac-Chg-Chg-Pro (4(R)-naphthalen-1-ylmethoxy)-OH (11g)

Described in example 3
11a

11b

11c →

11d

11e

15

69

11f   11g
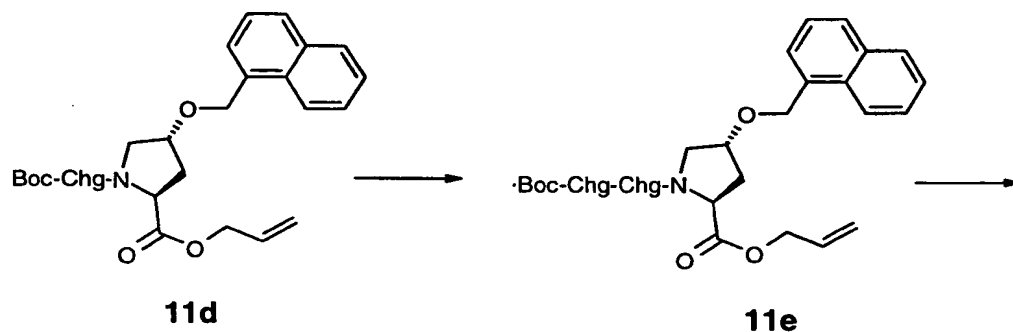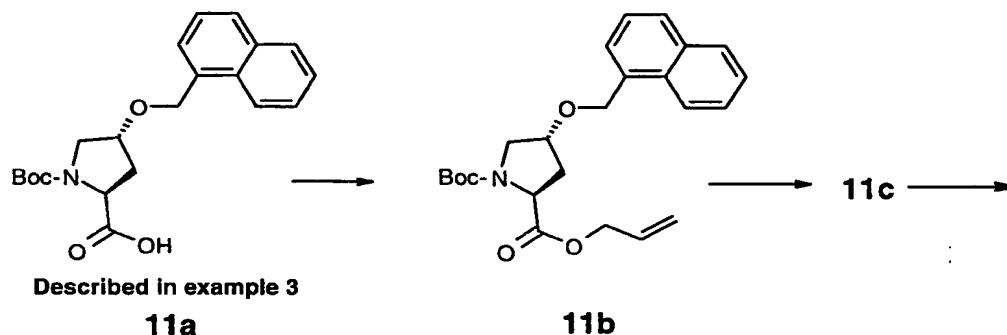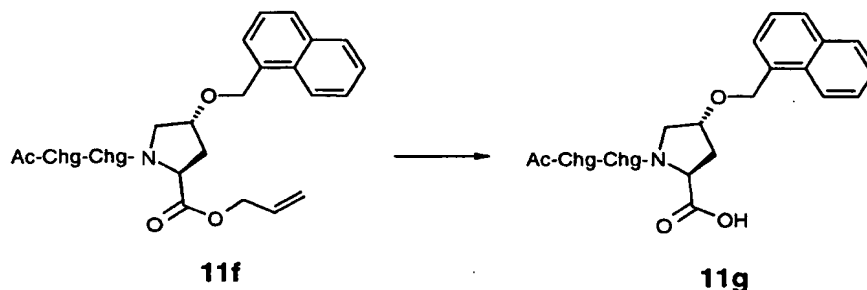
Compound **11a** (4.45g , 11.98 mmol) was dissolved in
anhydrous CH₃CN (60 mL). DBU (2.2 mL , 14.38mmol)
and allyl bromide (1.1mL , 13.18 mmol) were added
5   successively and the reaction mixture was stirred 24
h at RT The mixture was concentrated, the resulting
oil was diluted with EtOAc and water and successively
washed with water (2x) and brine (1x). The EtOAc
layer was dried (MgSO₄), filtered and evaporated to
10   dryness. The yellow oil was purified by flash
chromatography (eluent:hexane:EtOAc;90:10 to 85:15)
to provide the product **11b** as a yellow oil (2, 4.17g
; 85 % yield). MS (FAB)   412 MH⁺
¹H NMR (CDCl₃) , mixture of rotamers ca.1:2 , δ (d, J=
15   8Hz, 1H), 7.87 (d, J= 8Hz, 1H), 7.82 (d, J= 8Hz, 1H),
7.55-7.41 (m, 4H), 5.95-5.85 (m, 1H), 5.34-5.21 (m,
2H), 5.03-4.88 (m, 2H), 4.70-4.56 (m, 2H), 4.48 &
4.39 (t, J= 8, 15Hz, 1H), 4.28-4.23 (m, 1H), 3.81-
3.55 (m, 2H), 2.46-2.36 (m, 1H), 2.13-2.05 (m, 1H),
20   1.44 &1.41 (s, 9H).

Compound **11b** (2.08 g , 5.05 mmol) was treated for 30
min at RT with 4N HCl / dioxane. Evaporation to
dryness provided the corresponding amine-HCl as an
25   oil. The amine-HCl **11c** was dissolved in anhydrous DCM
(25 mL), NMM (2.2 mL, 20.22 mmol), Boc-Chg-OH · H₂O
(1.53 g, 5.56 mmol) and TBTU (1.95 g, 6.07 mmol) were
added successively. The reaction mixture was stirred
at RT overnight, then, diluted with EtOAc and

successively washed with 10% aqueous citric acid
(2x), saturated aq. NaHCO₃ (2x), water (2x), and
brine (1x). The EtOAc layer was dried (MgSO₄),
filtered and evaporated to dryness to provide the
5   crude product **11d** as a yellowish-white foam (ca 2.78
g, 100% yield). MS (FAB) 551.4 MH⁺. ¹H NMR (CDCl₃)
δ 8.03(d, J= 8Hz, 1H), 7.86 (b d, J= 8.5Hz, 1H), 7.84
(d, J= 8Hz, 1H), 7.56-7.40 (m, 4H), 5.92-5.85 (m,
1H), 5.31 (dd, J= 1, 17Hz, 1H), 5.22 (dd, J= 1, 10Hz,
10   1H), 5.17 (d, J= 9Hz, 1H), 5.05 (d, J= 12Hz, 1H),
4.91 (d, J= 12Hz, 1H), 4.67-4.60 (m, 3H), 4.31-4.27
(m, 2H), 4.16 (b d, J= 11Hz, 1H), 3.71 (dd, J= 4,
11Hz, 1H), 2.47-2.41 (m, 1H), 2.08-1.99 (m,1H), 1.85-
1.63 (m, 5H), 1.44-1.40 (m, 1H), 1.36 (s, 9H), 1.28-
15   1.00 (m, 5H).

The crude dipeptide **11d** (ca. 5.05 mmol) was treated
with 4N HCl/dioxane (25 mL) as described for compound
11c. The crude hydrochloride salt was coupled to
20   Boc-Chg-OH · H₂O (1.53g, 5.55 mmol) with NMM (2.22
mL, 20.22 mmol) and TBTU (1.95 g, 6.07 mmol) in DCM
(25 mL) as described for compound **11d** to yield crude
tripeptide as a yellow-oil foam. The crude material
was purified by flash chromatography
25   (eluent:hexane:EtOAc;80:20 to 75:25) to provide the
tripeptide **11e** as a white foam (2.75g ; 79% yield
over 2 steps). MS (FAB) 690.5 MH⁺. ¹H NMR (CDCl₃),
mainly one rotamer, δ 8.06 (d, J= 8Hz, 1H), 7.87 (b
d, J= 8.5Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.57-7.40 (m,
30   4H), 6.41 (d, J= 8.5Hz, 1H), 5.92-5.84 (m, 1H), 5.31
(dd, J= 1, 17Hz, 1H), 5.23 (dd, J= 1, 10.5Hz, 1H),
5.04 (d, J= 12Hz, 1H), 4.98 (b d, J= 7Hz, 1H), 4.93
(d, J=12Hz, 1H), 4.63-4.58 (m, 4H), 4.29-4.25 (m,
1H), 4.10-4.07 (m, 1H), 3.90-3.84 (m, 1H), 3.72 (dd,

J= 4, 11Hz, 1H), 2.48-2.40 (m, 1H), 2.07-1.99 (m,
1H), 1.83-1.55 (m, 12H), 1.43 (s, 9H), 1.23-0.89 (m,
10H)

5   The tripeptide **11e** (2.75 g, 3.99 mmol) was treated
with 4N HCl/dioxane (20 mL) as described for compound
11c. The crude hydrochloride salt was dissolved in
anhydrous DCM (20 mL). NMM (1.75 mL, 15.94 mmol) and
acetic anhydride (752 μL, 7.97 mmol) were added

10   successively. The reaction mixture was stirred
overnight at RT, then diluted with EtOAc. The organic
layer was washed successively with 10% aqueous
citric acid (2x), saturated aq. $NaHCO_3$ (2x), water
(2x) and brine (1x), dried ($MgSO_4$), filtered, and

15   evaporated to dryness to provide the crude tripeptide
11f as a white foam (2.48 g, 98% yield).
MS (FAB) 632.4 MH^{+1}. ^1H NMR ($CDCl_3$), mainly one
rotamer, δ 8.06(b d, J= 8Hz, 1H), 7.87 (b d, J= 8Hz,
1H), 7.83 (d, J= 8Hz, 1H), 7.58-7.40 (m, 4H), 6.36

20   (d, J= 9Hz, 1H), 6.01 (d, J= 9Hz, 1H), 5.94-5.83 (m,
1H), 5.34-5.28 (m, 1H), 5.25-5.21 (m, 1H), 5.05 (d,
J= 12Hz, 1H), 4.94 (d, J= 12Hz, 1H), 4.64-4.57 (m,
·4H), 4.30-4.23 (m, 2H), 4.12-4.08 (m, 1H), 3.73 (dd,
J= 4, 11Hz, 1H), 2.49-2.42 (m, 1H), 2.08-2.01 (m,

25   1H), 1.99 (s, 3H), 1.85-1.53 (m, 11H), 1.25-0.88 (m,
11H).

The crude tripeptide **11f** (2.48 g, 3.93 mmol)was
dissolved in an anhydrous mixture of CH_3CN : DCM (20

30   mL). Triphenylphosphine (53.5 mg, 0.200 mmol) and
tetrakis(triphenylphosphine)-palladium (0) catalyst
(117.9 mg, 0.102 mmol) were added successively,
followed by pyrrolidine (353.9 μL, 4.24 mmol). The
reaction mixture was stirred at room temperature for

18 h. Thereafter, the solvent was evaporated. The
residue was dissolved in EtOAc and 10% aqueous citric
acid , then, further washes twice more with 10%
aqueous citric acid, water (2x), and brine (1x). The

5   organic layer was dried (MgSO₄), filtered and
evaporated. The crude product was triturated in Et₂O:
DCM (85:15) to provide after filtration the
tripeptide **11g** as a white solid (2.09 g, 90% yield).
MS (FAB) 592.4 MH⁺   614.3 (M+Na) ⁺. ¹H NMR (CDCl₃),

10   mainly one rotamer, δ 8.08 (d, J= 8Hz, 1H), 7.93 (b
d, J= 9Hz, 1H),   7.88 (b d, J= 8Hz, 1H), 7.82 (d, J=
8Hz, 1H), 7.57-7.41 (m, 4H), 6.47 (d, J= 8.5Hz, 1H),
5.05 (d, J= 12.5Hz, 1H), 4.94 (d, J= 12.5Hz, 1H),
4.73 (t, J= 9.5, 19Hz, 1H), 4.44-4.35 (m, 2H), 4.26

15   (b s, 1H), 4.19 (d, J= 11.5Hz, 1H), 3.75 (dd, J= 4,
11Hz, 1H), 2.47 (b dd, J= 7.5, 13.5Hz, 1H), 2.20-2.11
(m, 1H), 2.04 (s, 3H), 1.88-1.41 (m, 11H), 1.30-0.80
(11H).

20   **Example 12**

**Synthesis of "tripeptide segment" -Ac-Chg-Val-
Pro(4(R)-naphthalen-1-ylmethoxy)-OH (12e)**

12a   12b

12c   12d

73
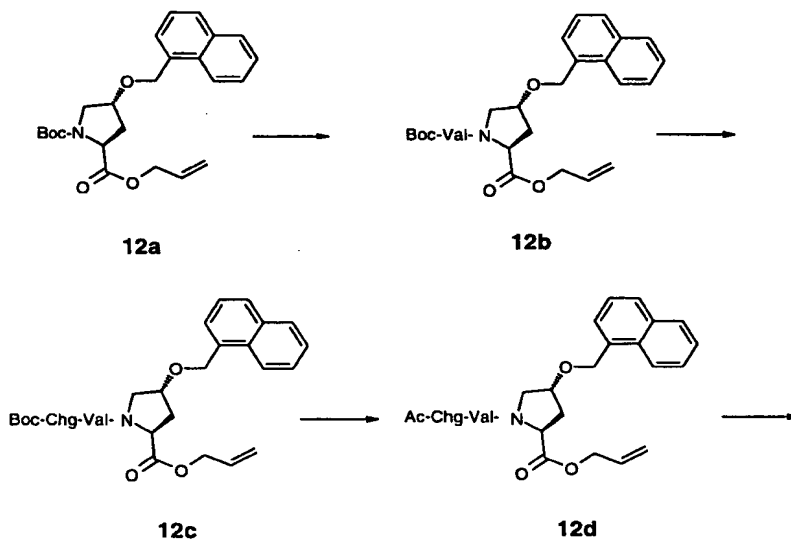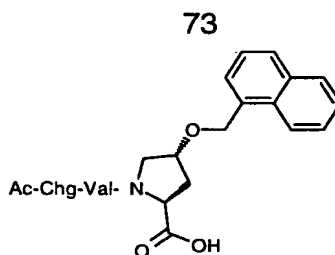
12e

Compound **12a** (2.89 g, 7.02mmol) was treated with 4N
HCl/dioxane (30 mL) as described for compound **11c**.
The crude hydrochloride salt was coupled to Boc-Val-
5   OH (1.53 g, 7.73 mmol) with NMM (3.1 mL, 28.09 mmol)
and TBTU (2.71 g, 8.43 mmol) in DCM (35 mL) for 3.5 h
as described for compound **3** to provide the crude
dipeptide **12b** as an ivory oil-foam (ca. 3.60 g, 100%
yield). MS (FAB) 509.3 MH⁻   511.3 MH⁺ 533.2
10 (M+Na)⁺. ^{1}H NMR (CDCl$_3$) δ 8.04 (b d, J= 8Hz, 1H),
7.87 (b d, J= 7Hz, 1H), 7.82 (d, J= 8Hz, 1H), 7.56-
7.40 (m, 4H), 5.93-5.85 (m, 1H), 5.34-5.28 (m, 1H),
5.24-5.19 (m, 2H), 5.04 (d, J= 12Hz, 1H), 4.92 (d, J=
12Hz, 1H), 4.67-4.60 (m, 3H), 4.31-4.26 (m, 2H),
15 4.11-4.09 (m, 1H), 3.72 (dd, J= 4, 11Hz, 1H), 2.48-
2.41 (m, 1H), 2.07-1.99 (m, 1H), 1.44-1.36 (m, 1H),
1.37 (s, 9H), 1.01 (d, J= 7Hz, 3H), 0.93 (d, J= 7Hz,
3H)

20 The crude dipeptide **12b** (ca. 7.02 mmol) was treated
with 4N HCl/dioxane (30 mL) as described for compound
11c. The crude hydrochloride salt was coupled to
Boc-Chg-OH · H$_2$O (2.13g , 7.73mmol) with NMM (3.1
mL, 28.09 mmol) and TBTU (2.71 g, 8.43 mmol) in
25 CH$_2$Cl$_2$ (35 mL) as described for compound 3 to provide
the crude tripeptide **12c** as an ivory foam (ca. 4.6 g,
100% yield). MS (FAB) 648.5 MH⁻   672.4 (M+Na) ⁺.
^{1}H NMR (CDCl$_3$) δ 8.06 (b d, J=8Hz, 1H), 7.87 (b d, J=

7.5 Hz, 1H), 7.82 (b d , J= 8Hz, 1H), 7.57-7.40 (m, 4H), 6.46 (b d, J= 8.5Hz, 1H), 5.94-5.84 (m, 1H), 5.31 (dd, J= 1, 17Hz, 1H), 5.23 (dd, J= 1, 10.5Hz, 1H), 5.03 (d, J= 12Hz, 1H), 5.00-4.97 (m, 1H), 4.93 (d, J=, 12Hz, 1H), 4.63-4.59 (m, 4H), 4.29-4.27 (m, 1H), 4.10-4.07 (m, 1H), 3.92-3.86 (m, 1H), 3.72 (dd, J= 5, 11Hz, 1H), 2.48-2.41 (m, 1H), 2.10-1.99 (m, 1H), 1.76-1.57 (m, 6H), 1.43 (s, 9H), 1.20-0.92 (m, 6H), 1.00 (d, J= 7Hz, 3H), 0.93 (d, J= 7Hz, 3H).

The crude tripeptide **12c** (ca. 7.02mmol) was treated with 4N HCl/dioxane (30 mL) as described for compound **11c**. The crude hydrochloride salt was further treated with acetic anhydride (1.33 mL, 14.05 mmol) and NMM (3.1 mL, 28.09 mmol) in CH_2Cl_2 (35 mL) as described for compound **11f**. The crude product was flash purified (eluent:hexane:EtOAc;30:70) to provide the acetylated protected tripeptide **12d** as a white foam (3.39 g, 81% yield over 3 steps). MS (FAB) 590.3 MH⁻ 592.4 MH⁺ 614.4 (M+Na)⁺

^{1}H NMR (CDCl$_3$), mainly one rotamer, δ 8.06 (d, J= 8Hz, 1H), 7.88 (b d, J= 8Hz, 1H), 7.83 (d, J= 8Hz, 1H), 7.58-7.41 (m, 4H), 6.37 (d, J= 9Hz, 1H), 5.97 (d, J= 8.5 Hz, 1H), 5.94-5.84 (m, 1H), 5.31 (dd, J= 1, 17Hz, 1H), 5.24 (dd, J= 1, 10.5 Hz, 1H), 5.05 (d, J= 12Hz, 1H), 4.94 (d, J= 12Hz, 1H), 4.66-4.57 (m, 4H), 4.31-4.22 (m, 2H), 4.11-4.05 (m, 1H), 3.73 (dd, J= 4.5, 11Hz, 1H), 2.50-2.43 (m, 1H), 2.09-2.01 (m, 2H), 2.00 (s, 3H), 1.68-1.55 (m, 5H), 1.15-0.89 (m, 6H), 0.99 (d, J= 7Hz, 3H), 0.91 (d, J= 7Hz, 3H).

The acetylated tripeptide **12d** (3.39 g, 5.73 mmol) was deprotected by tetrakis(triphenylphosphine)-palladium (0) catalyst (172.1 mg, 0.149 mmol) with

triphenylphosphine (78.1 mg, 0.298 mmol) and
pyrrolidine (516 μL, 6.19 mmol) in a 1:1 mixture of
anhydrous CH_3CN : DCM (30 mL) as described for
compound **11g**. The crude light yellow foam product was
trichurated in Et_2O : DCM (85:15)to provide after
filtration the tripeptide **12e** as an off-white solid
(3.0 g ; 95% yield). MS (FAB)   550.3 MH^-
^1H NMR ($CDCl_3$) δ 8.08 (d, J= 8Hz, 1H), 8.04 (b d, J=
9Hz, 1H), 7.88 (b d, J= 7.5Hz, 1H), 7.82 (d, J= 8Hz,
1H), 7.58-7.37 (m, 5H), 5.05 (d, J= 12Hz, 1H), 4.94
(d, J= 12Hz, 1H), 4.61 (t, J= 9.5, 19.5Hz, 1H), 4.46-
4.37 (m, 2H), 4.27 (b s, 1H), 4.17 (d, J= 11Hz, 1H),
3.74 (dd, J= 4, 11Hz, 1H), 2.49 (b dd, J= 7.5, 13Hz,
1H), 2.17-2.09 (m, 1H), 2.04 (s, 3H), 2.03-1.94 (m,
1H), 1.79 (b d, J= 12.5Hz, 1H), 1.62-1.43 (m, 5H),
1.08-0.85 (m, 5H), 1.00 (d, J= 7Hz, 3H), 0.90 (d, J=
7Hz, 3H).

Example 13

**General procedure for coupling reactions done on
solid support.**

The synthesis was done on a parallel synthesizer
model ACT396 from Advanced ChemTech® with the 96 well
block. Typically, 24 peptides were synthesized in
parallel using standard solid-phase techniques. The
starting Fmoc-Nva-Wang resin and the 1-(Fmoc-
amino)cyclopropane carboxylic acid-Wang resin were
prepared by the DCC/DMAP coupling method (Atherton,
E; Scheppard, R.C. *Solid Phase Peptide Synthesis, a
Practical Approach*; IRL Press: Oxford (1989); pp 131-
148). Other amino acid-Wang resins were obtained
from commercial sources.

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Each well was loaded with 100 mg of the starting
resin (approximately 0.05 mmol). The resins were
washed successively with 1.5 mL portions of NMP (1 X)
and DMF (3 X). The Fmoc protecting group was removed
5   by treatment with 1.5 mL of a 25% v/v solution of
piperidine in DMF for 20 min. The resins were washed
with 1.5 mL portions of DMF (4 X), MeOH (3 X) and DMF
(3 X). The coupling was done in DMF (350 μL), using
400 μL (0.2 mmol) of a 0.5M solution of Fmoc-amino
10   acid/HOBt hydrate in DMF, 400 μL (0.4 mmol) of a 0.5M
solution of DIPEA in DMF and 400 μL (0.2 mmol) of a
0.5M solution of TBTU in DMF. After shaking for 1 h,
the wells were drained, the resins were washed with
1.5 mL of DMF and the coupling was repeated once more
15   under the same conditions. The resins were then
washed as described above and the cycle was repeated
with the next amino acid.

The capping groups were introduced in two ways:
20   1. In the form of a carboxylic acid using the
protocol·described above (for example acetic acid)
or,
2. As an acylating agent such as an anhydride or an
acid chloride. The following example illustrates the
25   capping with succinic anhydride: After the Fmoc
deprotection and subsequent washing protocol, DMF was
added (350 μL), followed by 400 μL each of a DMF
solution of succinic anhydride (0.5 M, 0.2 mmol) and
DIPEA (1.0 M, 0.4 mmol). The resins were stirred for
30   2 h and a recoupling step was performed.

At the end of the synthesis the resin was washed with
1.5 mL portions of DCM (3 x), MeOH (3 x), DCM (3 x),
and were dried under vacuum for 2 h.

The cleavage from the resin and concomitant side
chain deprotection was effected by the addition of
1.5 mL of a mixture of TFA, H_2O, DTT and TIS (92.5:
2.5: 2.5: 2.5). After shaking for 2.5 h, the resin
was filtered and washed with 1.5 mL of DCM. The
filtrates were combined and concentrated by vacuum
centrifugation.

Each compound was purified by preparative reversed
phase HPLC using a C18 column (22 mm by 500 mm). The
product-containing fractions were identified by
MALDI-TOF mass spectrometry, combined and
lyophilized.

**Example 14**

Synthesis of compound 210 (Table 2)
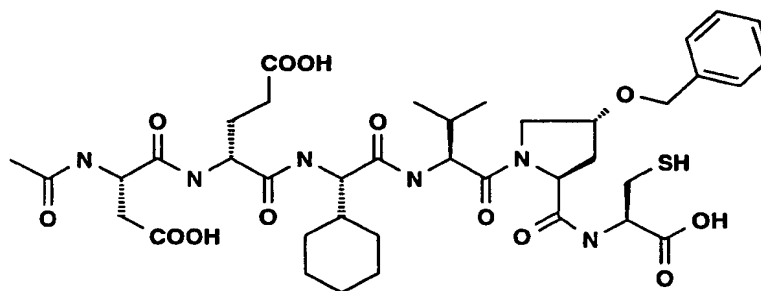
210

Using the experimental protocol described in Example
11 and starting with Fmoc-Cys(Trityl)-Wang resin, the
above compound was obtained as a white solid (15.7
mg). MS (FAB) 849.2 (MH+), ^{1}H NMR (DMSO-d_6) δ 12.8
(broad s, 1H), 12.1 (broad s, 2H), 8.27 (d, J = 8 Hz,
1H), 8.17 (d, J = 7.5 Hz, 1H), 8.07 (d, J = 8 Hz,
1H), 8.00 (d, J = 8.4 Hz, 1H), 7.75 (d, J = 8.9 Hz,
1H), 7.34-7.27 (m, 5H), 4.54-4.39 (m, 5H), 4.31-4.18
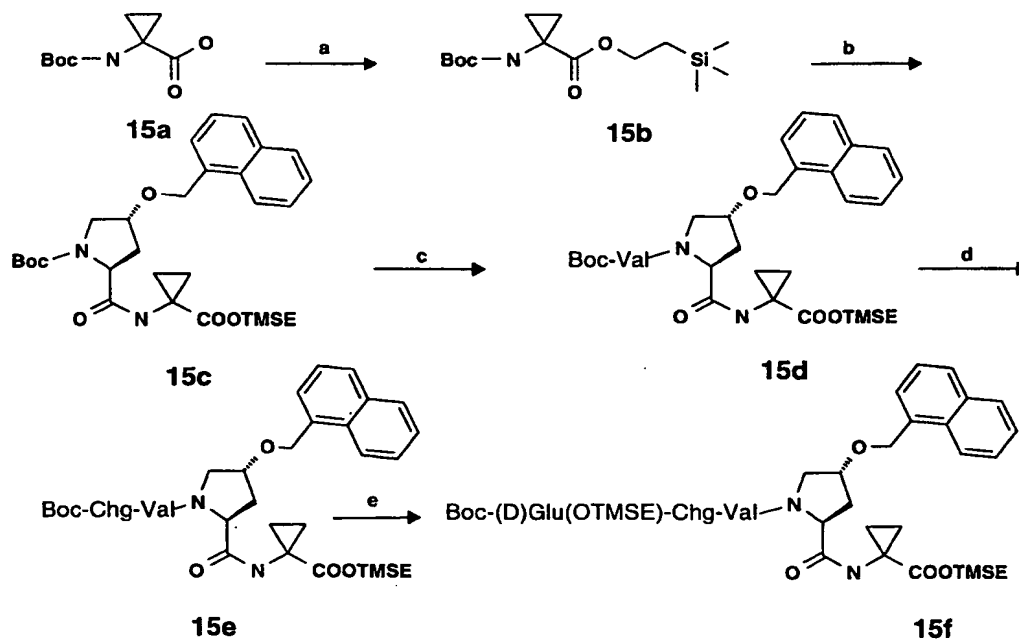(m, 4H), 4.10 (d, J = 11 Hz, 1H), 3.68 (dd, J = 3.9

Hz, J' = 10.8 Hz, 1H), 2.90-2.82 (m, 1H), 2.78-2.70
(m, 1H), 2.67-2.42 (m, 4H), 2.21-2.17(m, 3H), 2.00-
1.85 (m, 3H), 1.83 (s, 3H), 1.80-1.67 (m, 1H), 1.67-
1.42 (m, 6H), 1.15-0.95 (m, 4H), 0.88 (dd, J = 6.9
Hz, J' = 8.9 Hz, 6H).

Example 15

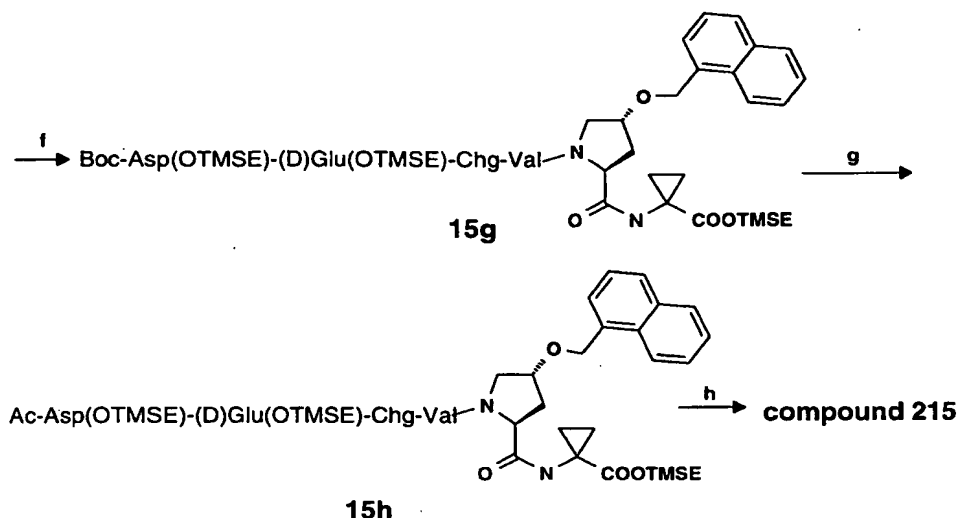**Synthesis of compound 215 (Table 2)**

:
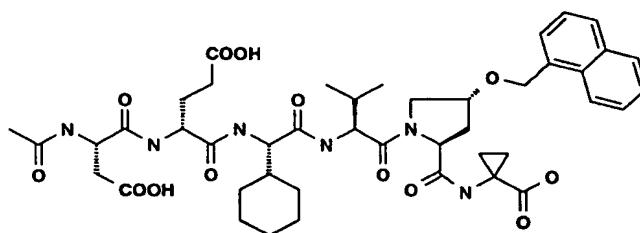
215

The synthesis was carried out as shown below:

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15g

Ac-Asp(OTMSE)-(D)Glu(OTMSE)-Chg-Val-

15h

a) Synthesis of compound 15b:

1-(N-t-Boc-amino)cyclopropanecarboxylic acid (**15a**) (997 mg, 4.96 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (25 mL) and THF (10 mL) . The solution was cooled to 0°C , 2-trimethylsilylethanol (0.852 mL , 5.95 mmol), DMAP (121.1 mg, 0.991 mmol) and a DCC/CH_2Cl_2 solution (3.65 M; 1.63 mL, 5.95 mmol) were added successively. The reaction mixture was stirred at 0°C for ca.4 h then at RT overnight. The white suspension was filtered through a diatomaceous earth pad. The pad was and rinsed with CH_2Cl_2. Filtrate and washing were evaporated to dryness. The residue was diluted with EtOAc and sequentially washed with 10% aqueous citric acid (2x), saturated $NaHCO_3$ (2x), water (2x) and brine (1x). The organic layer was dried ($MgSO_4$), filtered, and evaporated to provide ester **15b** as an oil (ca.1.5 g, 100%). ^1H NMR ($CDCl_3$) δ 5.08 (s, 1H), 4.20-4.16 (m, 2H), 1.57-1.43 (m, 2H), 1.45 (s , 9H), 1.17-1.12 (m, 2H), 1.00-0.94 (m, 2H), 0.04 (s, 9H).

b) Synthesis of compound 15c:

Ester **15b** (ca.700 mg, 2.33 mmol) was treated for 40
min at RT with 4N HCl/dioxane (11 mL). The solution
was concentrated to dryness to provide the amine
hydrochloride as a white solid which was then
subjected to the reaction conditions described in
Example 6. The crude hydrochloride salt (950 mg,
2.55 mmol) and Boc-4*(R)*-(naphthalen-1-
ylmethoxy)proline (**3**) were dissolved in anhydrous
CH_2Cl_2. NMM (1.02 mL, 9.30 mmol) and HATU (1.06 g,
2.79 mmol) were added successively and the mixture
was stirred at RT. After 1.75 h, the reaction
mixture was diluted with EtOAc and washed
sequentially with 10% aq. citric acid (2x), saturated
aq. $NaHCO_3$ (2x), water (2x), and brine(1x). The EtOAc
layer was dried ($MgSO_4$), filtered and concentrated to
dryness to provide the crude dipeptide **15c** as an off-
white foam (1.22 g). MS (FAB) 555.4 (MH^+). ^1H NMR
($CDCl_3$) ; mixture of rotamers, δ   8.06-8.04 (m,
1H), 7.87-7.80 (m, 2H), 7.55-7.41 (m, 5H), 4.99-4.93
(m, 2H), 4.45-4.21 (m, 2H), 4.16-4.11 (m, 2H), 3.97-
3.45 (m, 2H), 2.70-1.80 (m, 2H), 1.73-1.40 (m, 2H),
1.53 (s, (6/9) 9H), 1.44 (s, (3/9) 9H), 1.20-1.05 (m
, 2H), 0.97-0.93 (m, 2H), 0.02 (s, 9H).

c) Synthesis of compound 15d:

The crude dipeptide **15d** (ca. 2.20 mmol) was treated
with 4N HCl/dioxane (11 mL) 40 min, RT and the
resulting hydrochloride salt was coupled to Boc-Val-
OH (525 mg, 2.42 mmol) with NMM (968 mL, 8.80 mmol)
and HATU (1.00 g, 2.64 mmol) as described for
compound **15c** (with the modification of 2.5 h coupling
time). The crude tripeptide **15d** was obtained as an
off-white foam (1.5 g). MS (FAB) 654.4 (MH^+). ^1H

NMR (CDCl$_3$) δ 8.05-8.02 (m, 1H), 7.87-7.80 (m, 2H), 7.55-7.40 (m, 5H), 7.30-7.28 (m, 1H), 5.19-4.62 (m, 4H), 4.41-3.70 (m, 1H), 4.35-4.27 (m, 1H), 4.09-3.95 (m, 1H) 3.73-3.62 (m, 2H), 2.69-2.60 (m, 1H) , 2.14-

5   1.94 (m, 2H), 1.55-1.38 (m, 2H), 1.39 (s, 9H), 1.22-1.18 (m, 1H), 1.11-1.07 (m, 1H), 0.98-0.90 (m, 8H), 0.02 (s, 9H).

d) Synthesis of compound 15e:

10 The crude tripeptide **15d** (ca. 2.20 mmol) was treated with 4N HCl/dioxane (11 mL) 40 min, RT and the resulting hydrochloride salt was coupled to Boc-Chg-OH (622 mg, 2.42 mmol) with NMM (968 mL, 8.80 mmol) and TBTU (847 mg, 2.64 mmol) as described for

15 compound **15c** (with the modifications of using TBTU as a coupling agent and stirring at RT for ca. 64 h prior to work-up). The foam-like residue was purified by flash chromatography (eluent: hexane: EtOAc; 6:4) to provide the tetrapeptide **15e** as a white foam

20 (710.8 mg ; 41% yield over 3 steps). MS (FAB) 793.4 (MH$^+$). ^{1}H NMR (CDCl$_3$) δ 8.07-8.05 (m, 1H), 7.87-7.80 (m, 2H), 7.57-7.41 (m, 4H), 7.35 (s, 1H), 6.72-6.64 (m, 1H), 5.02-4.95 (m, 3H), 4.68-4.62 (m, 2H), 4.43-4.40 (m, 1H), 4.15-4.00 (m, 2H), 3.96-3.93 (m, 2H), 3.68 (dd, J= 11, J'= 5 Hz, 1H), 2.62-2.56 (m,

25 2H), 3.68 (dd, J= 11, J'= 5 Hz, 1H), 2.62-2.56 (m, 1H), 2.16-2.00 (m, 2H), 1.70-1.54 (m, 6H), 1.49-1.42 (m, 2H), 1.43 (s, 9H), 1.14-1.02 (m, 5H), 0.95-0.88 (m, 10H), 0.02 (s, 9 H).

e) Synthesis of compound 15f:

30 Tetrapeptide **15e** (168.1 mg, 0.212 mmol) was treated with 4N HCl/dioxane solution (2 mL) and the resulting hydrochloride salt was coupled to Boc-(D)Glu(OTMSE)-OH (81.0 mg, 0.233 mmol) with NMM (94 mL, 0.848 mmol)

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and TBTU (81.7 mg, 0.254 mmol) as described for
compound **15e** (with the modification of 17 h coupling
time). The crude pentapeptide **15f** was obtained as an
off-white foam (220 mg, 0.212 mmol) . MS (FAB)
5   1022.8 (MH^+) 1044.8 (MNa^+). ^1H NMR ($CDCl_3$) δ 8.07-8.05
(m, 1H), 7.88-7.81 (m, 2H), 7.57-7.41 (m, 4H), 7.29
(s, 1H), 6.70-6.55 (m, 2H), 5.45-5.35 (m, 1H), 4.99-
4.98 (m, 2H) , 4.66-4.57 (m, 2H), 4.44-4.40 (m, 1H),
4.30-4.01 (m , 5H), 3.91 (dd, J= 11, J'= 4 Hz, 1H),
10   3.76-3.62 (m, 2H), 2.62-2.56 (m , 1H), 2.50-2.30 (m,
3H), 2.18-2.09 (m, 2H), 2.06-1.90 (m, 2H), 1.67-1.53
(m, 4H), 1.50-1.42 (m, 4H), 1.43 (s, 9H) , 1.14-0.86
(m, 10H), 0.93 (d, J= 7 Hz, 3H), 0.87 (d, J= 7 Hz,
3H), 0.04 (s, 9H), 0.02 (s, 9H).

15

f) Synthesis of compound 15g:

The crude pentapeptide **15f** (ca. 0.212 mmol) was
treated with 4N HCl/dioxane solution (2.5 mL) 40 min,
RT and the resulting hydrochloride salt was coupled
20   to Boc-Asp(OTMSE)-OH (77.8 mg, 0.233 mmol) with NMM
(93 mL, 0.848 mmol) and TBTU (81.7 mg, 0.254 mmol) as
described for compound **15e** (with the modification of
2.5 h coupling time). The crude hexapeptide **15g** was
obtained as an ivory foam (278 mg, 0.212 mmol). MS
25   (FAB)   1237.5 (MH^+)   1259(MNa^+).

g) Synthesis of compound 15h:

The crude hexapeptide **15g** (ca. 0.2 mmol) was treated
for 40 min at RT with 2.5 mL 4N HCl/dioxane solution.
30   Concentration to dryness provided the amine
hydrochloride as a white solid. The crude
hydrochloride salt was dissolved in anhydrous DMF
(2.5 mL) and treated successively with pyridine (377
μL, 4.66 mmol) and acetic anhydride (378 μL, 4.01

mmol). The reaction mixture was stirred overnight at
RT then poured into brine and extracted with EtOAc
(3x). The combined organic layer was washed
successively with 10% aqueous citric acid (2x),

5   saturated NaHCO₃ (2x), water (2x), and brine (1x).
The organic layer was dried (MgSO₄), filtered and
evaporated to dryness. The foamy residue was purified
by flash chromatography (eluent : hexane : EtOAc;
3:7) to provide the acetylated hexapept **15h** as an

10   off-white foam (78.5 mg, 31% yield over 3 steps). MS
(FAB) 1179.6 (MH⁺) 1201.5 (MNa⁺). ¹H NMR (CDCl₃) δ
8.11-8.09 (m, 1H), 7.86-7.79 (m, 2H), 7.55-7.41 (m,
5H), 7.28 (s, 1H), 7.02-6.96 (m, 2H), 6.70-6.68 (m,
1H), 5.13-5.10 (m, 1H), 4.96-4.91 (m, 2H), 4.58-4.41

15   (m, 4H), 4.22-4.08 (m, 8H), 3.77 (dd, J= 10.5, J'= 5
Hz, 1H), 3.09 (dd, J= 18, J'= 4 Hz, 1H), 2.76 (dd, J=
17.5, J'= 8 Hz, 1H), 2.51-2.20 (m, 3H), 2.12-2.08 (m,
2H), 2.09 (s, 3H), 1.73-1.53 (m, 8H), 1.27-1.09 (m,
7H), 1.01-0.85 (m, 8H), 0.98 (d, J= 6.5 Hz, 3H),

20   0.97(d, J= 6 Hz, 3H), 0.04 (s, 9H), 0.03 (s, 9H),
0.01 (s, 9H).

h) Synthesis of compound 215:

The acetylated hexapeptide **15h** (76.5 mg, 0.065 mmol)

25   was dissolved in anhydrous THF (2 mL), a TBAF
solution (1M in THF; 389 μL, 0.389 mmol) was added
and the mixture was stirred at RT for 16 h. The
solution was concentrated under vacuum and the
residue was dissolved in glacial acetic acid,

30   filtered through a Millipore®: Millex®-HV 0.45 μm
filter unit and injected onto an equilibrated Whatman
Partisil® 10-ODS-3 (2.2 x 50cm) C18 reverse phase
column. Purification program: Linear Gradient at 15

mL/min, λ 230 nm, program at 5% A for 10 min, 5-30% A
in 10 min, at 30% A for 10 min, 30-60% A in 90 min
A:0.06% TFA/CH₃CN; B:0.06% TFA/H₂O. Fractions were
analyzed by analytical HPLC. The product collected

5   was lyophilized to provide the hexapeptide acid **215**
as a white amorphous solid (26.9 mg; contains 41% by
weight of tetrabutylammonium salts, 28% yield). MS
(FAB) 879.4 (MH⁺) 901.3 (MNa⁺). In order to remove
the tetrabutylammonium salt, the above product (ca.18

10   mg) was dissolved in EtOAc and washed with 10% HCl
(2x). The EtOAc layer was evaporated, then
lyophilized with water to provide the salt -free
product as a white amorphous solid (3.8 mg , 36%
yield). ¹H NMR (DMSO-d₆) δ 8.39 (s, 1H), 8.10-7.81

15   (m, 7H), 7.57-7.45 (m, 4H), 5.07-4.87 (m, 2H), 4.55-
4.00 (m, 7H), 3.76-3.71 (m, 1H), 2.67-2.62 (m,1H),
2.33-2.10 (m, 3H), 2.05-1.42 (m, 8H), 1.79 (s, 3H) ,
1.38-0.71 (m, 1H), 0.89 (d, J= 6.68 Hz, 3H), 0.86 (d,
J=6.36 Hz, 3H).

20

Example 16

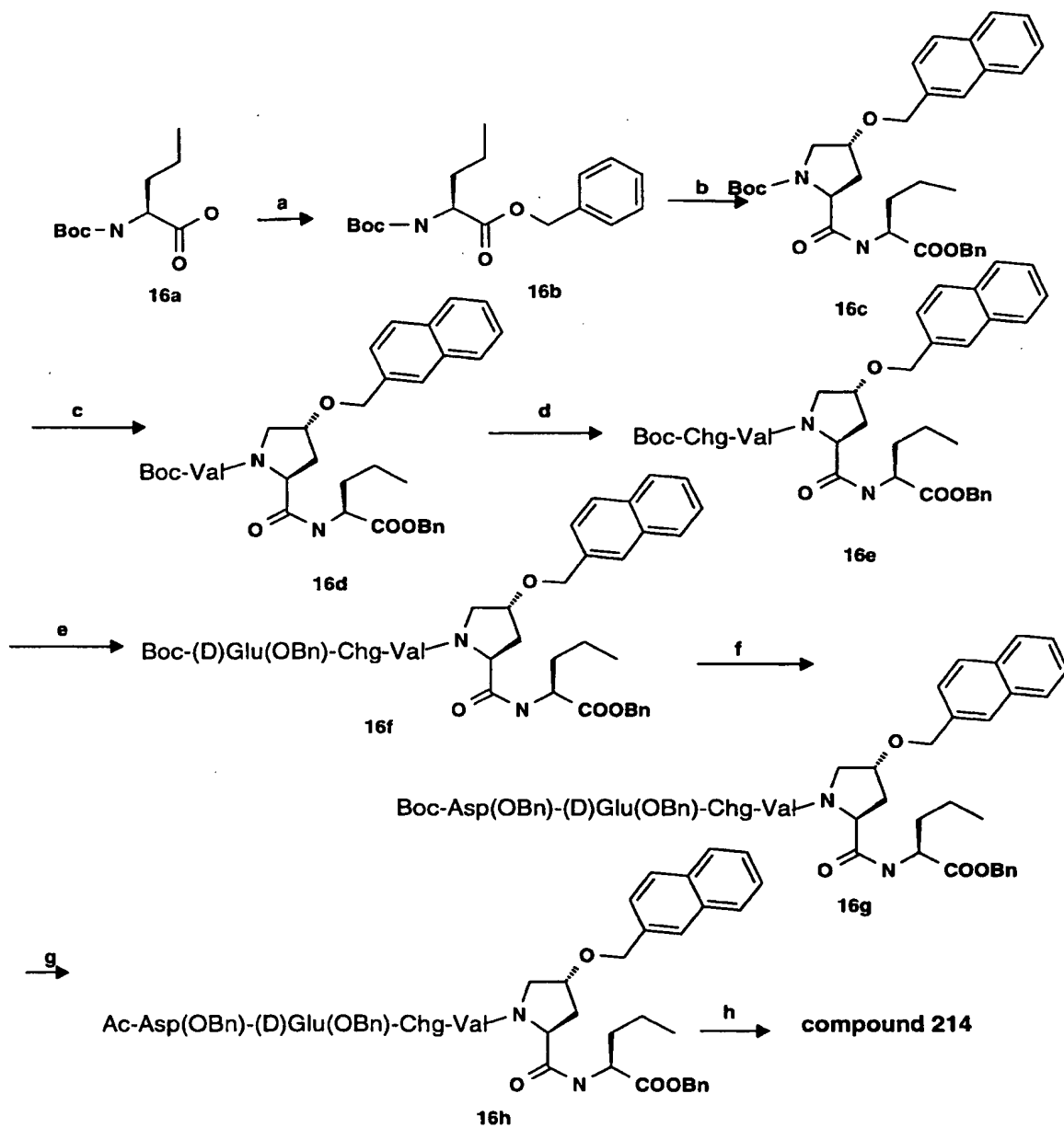**Synthesis of compound 214 (Table 2):**

214
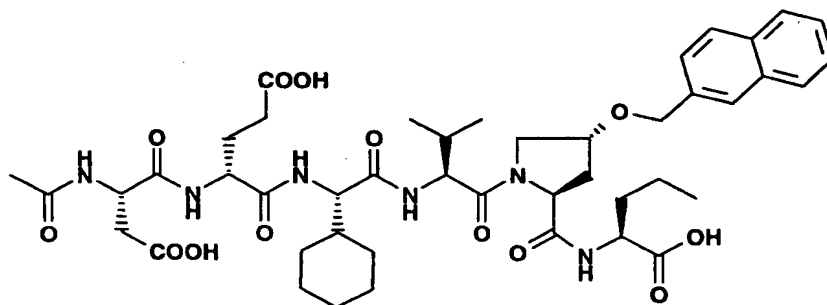
25   For the synthesis of compound **214** the procedure
described in example 15 was followed, using Boc-4*(R)*-
(naphthalen-2-ylmethoxy)proline for the introduction

of the P2 fragment and with different protecting
groups at the side chain carboxylic acid residues.

The synthesis is described below:

a) Synthesis of compound 16b:

At 0°C, benzyl bromide (5.74 mL, 48.3 mmol) was added to a mixture of Boc-norvaline (**16a**) (10.0 g, 46.0 mmol) and DBU (7.57 mL, 50.6 mmol) in acetonitrile

5   (200 mL). After stirring at RT for 20 h, the solution was concentrated and the residue dissolved in ether. The organic solution was washed sequentially with 10% aqueous citric acid (2x), saturated aqueous. NaHCO₃ (2x) and brine (1x), dried (MgSO₄), filtered and

10   concentrated to give the desired benzyl ester **16b** as a colorless oil (13.7 g, 97% yield). ^{1}H NMR (CDCl₃) δ 7.40-7.32 (m, 5H), 5.16 (dd, J = 26.7, J'= 12.4 Hz, 2H), 4.99 (d, J = 7.9 Hz, 1H), 4.35-4.32 (m, 1H), 1.82-1.73 (m, 1H), 1.66-1.57 (m, 1H), 1.43 (s, 9H),

15   1.41-1.32 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H).

b, c, d, e, f, g) Synthesis of compound 16h:

The above Boc-Nva benzyl ester (121 mg, 0.48 mmol) was subjected to the same sequence of reactions as

20   described in example 7. However, for the introduction of P2 (step b) Boc-4*(R)*-(naphthalen-2-ylmethoxy)proline was used. Also, for the introduction of P5 (step e) and P6 (step f) the corresponding Boc-D-Glu-OH and Boc-Asp-OH residues

25   were protected as benzyl esters at the carboxylic acid side chain.


h) Synthesis of compound 214:

To a solution of hexapeptide **16h** (ca. 0.210 mmol) in

30   ethanol (3 mL) was added 10% palladium on charcoal (10 mg) and ammonium acetate (10 mg). The mixture was stirred under an atmosphere of hydrogen for 5 h, then filtered through a Millipore®: Millex®-HV 0.45 μm filter unit and injected onto an equilibrated Whatman

Partisil® 10-ODS-3 (2.2 x 50 cm) C18 reverse phase
column. Purification program: Linear Gradient at 15
mL/min, λ 230 nm, at 5% to 50% A in 60 min   A: 0.06%
TFA/CH₃CN; B: 0.06% TFA/H₂O. Fractions were analyzed
by HPLC . The collected product was lyophilized to
provide **214** as a white solid (20 mg, 0.02 mmol). MS
(FAB) 895.5 (MH⁺). ¹H NMR (CDCl₃)   δ 8.16 (d, J =
7.6 Hz, 1H), 8.11 (d, J = 8 Hz, 1H), 8.09 (d, J = 8
Hz, 1H), 7.98 (d, J = 9 Hz, 1H), 7.91-7.88 (m, 3H),
7.85 (s, 1H), 7.77 (d, J = 9 Hz, 1H), 7.51-7.46 (m,
3H), 4.70 (d, J = 12 Hz, 1H), 4.60 (d, J = 12 Hz,
1H), 4.53-4.45 (m, 2H), 4.33-4.10 (m, 6H), 3.69 (dd,
J = 19, J'= 4.4 Hz, 1H), 2.66-2.60 (m, 1H), 2.49-2.43
(m, 1H), 2.21-2.18 (m, 3H), 2.07-1.94 (m, 3H), 1.82
(s, 3H), 1.76-1.33 (m, 10H), 1.04-0.86 (m, 15H).

Example 17

Synthesis of compound 221 (Table 2):
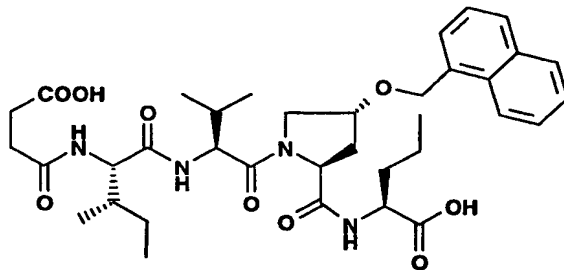
221

Mono-benzylsuccinic acid (prepared as described in:
Bischoff, V. et al., Chem.Ber. (1902), 35, 4078) (27
mg, 0.134 mmol) was stirred in acetonitrile (2 mL)
with TBTU (52 mg, 0.160 mmol) and NMM (47 mg, 0.469
mmol) for 5 min. To this mixture, the hydrochloride
salt of the appropriate tetrapeptide (prepared as
described for compound **16e** but using isoleucine
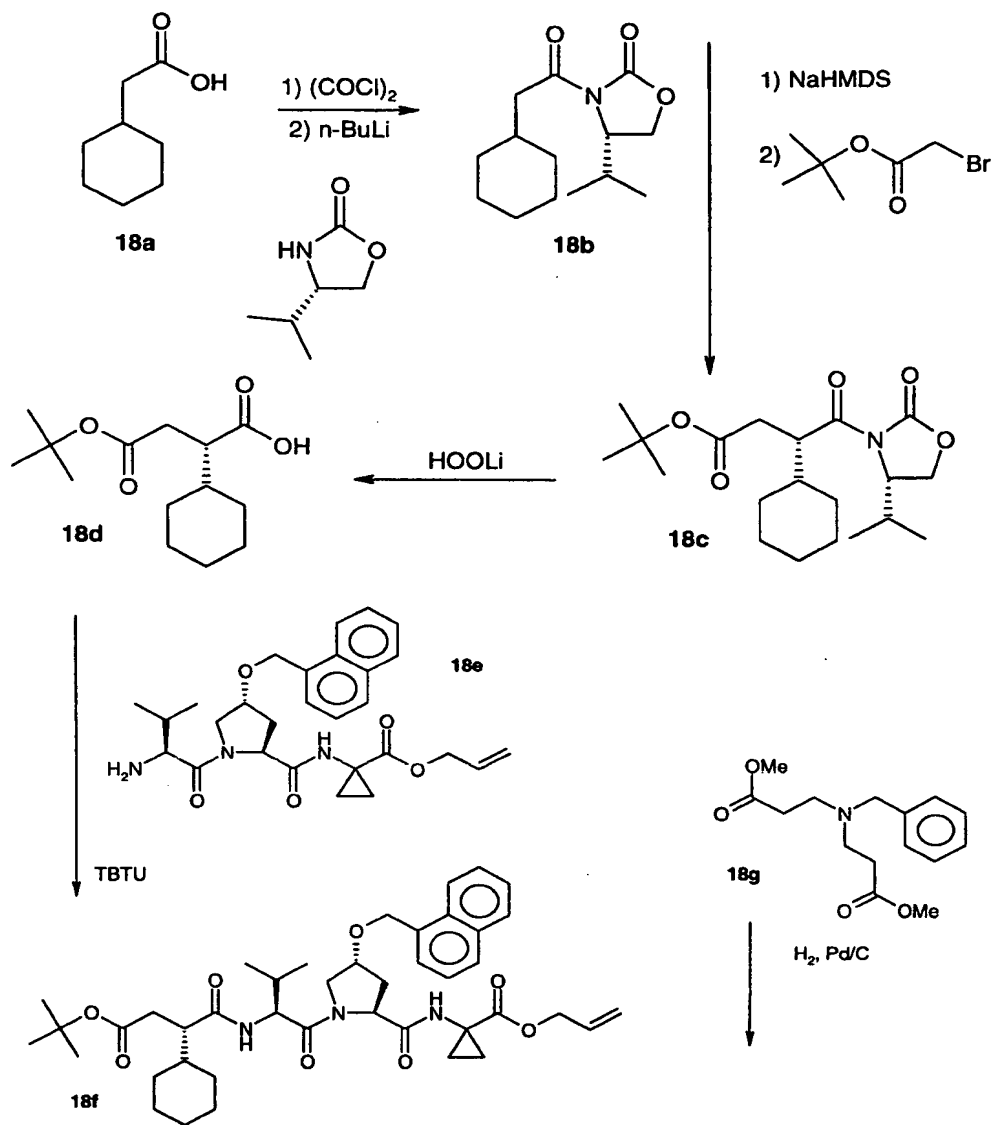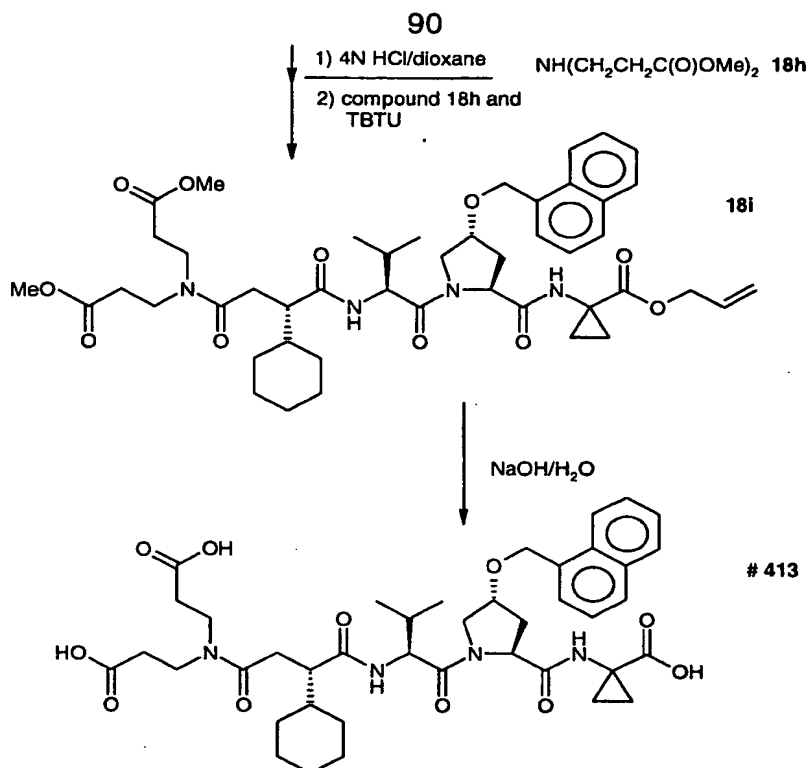instead of cyclohexylglycine and 4(R)-(naphthalen-1-

ylmethoxy)proline instead of a 4 *(R)*-(naphthalen-2-ylmethoxy)proline (97.0 mg, 0.134 mmol) was added. The mixture was stirred at RT for 2.5 h. Ethyl acetate was added and the mixture was washed with 10%

5 aqueous citric acid (2x), with saturated aqueous NaHCO$_3$ (2x) and brine (1x), dried (MgSO$_4$), filtered and concentrated to afford the protected tetrapeptide as a yellow oil.

10 The above compound (ca. 0.134 mmol) was dissolved in ethanol (3 mL) and ammonium acetate (10 mg) and 20% palladium hydroxide on activated carbon (30 mg) were added. The mixture was stirred under 1 atmosphere of hydrogen for 18 h, then filtered through a

15 Millipore®: Millex®-HV 0.45 μm filter unit and injected onto an equilibrated Whatman Partisil 10-ODS-3 (2.2 x 50 cm) C18 reverse phase column. Purification program: Linear Gradient at 15 mL/min, λ 230 nm, 5% A for 10 min, 5-60% A in 60 min (A:

20 0.06% TFA/CH$_3$CN; B: 0.06% TFA/H$_2$O). Fractions were analyzed by HPLC . The collected product was lyophilized to provide **221** as a white solid (21 mg). MS (FAB) 683 (MH$^+$). ^{1}H NMR (DMSO-d$_6$)   δ 8.12 (d, J = 7.6 Hz, 1H), 8.07-8.03 (m, 1H), 7.96-7.81 (m, 4H),

25 7.59-7.51 (m, 3H), 7.55 (t, J = 8.0 Hz, 1H), 4.90 (d, J =8 Hz, 1H), 4.82 (d, J = 8 Hz, 1H), 4.45 (t, J = 8.0 Hz, 1H), 4.36-4.31 (m, 2H), 4.24-4.12 (m, 3H), 3.74-3.68 (m, 1H), 2.43-2.31 (m, 4H), 2.24-2.18 (m, 1H), 2.01-1.92 (m, 2H), 1.67-1.51 (m, 3H), 1.42-1.32

30 (m, 3H), 1.14-0.96 (m, 1H), 0.93-0.67 (m, 15H).

Example 18

The following description is an example of a compounds of formula I wherein Q is CH$_2$C(O).

Preparation of compound **413** (Table 4)

90

1) 4N HCl/dioxane   NH(CH₂CH₂C(O)OMe)₂ **18h**

2) compound 18h and
   TBTU

[chemical structure diagram with label **18i**]

NaOH/H₂O

[chemical structure diagram with label **# 413**]

Compound 18b

5   1)   To cyclohexylacetic acid (**18a**) (8g, 56.25 mmol)
in DCM (160 mL) at room temperature was added the
oxalyl chloride (6.4 mL, 73.14 mmol) and 2 drops of
DMF. The reaction mixture was stirred at room
temperature for 1h, then concentrated under reduced
pressure to give cyclohexylacetyl chloride.

10   2)   The chiral auxiliary, (4S)-(-)-4-isopropyl-2-
oxazolidinone, (7.63g, 59.06 mmol) was dissolved in
THF (200 mL) and cooled to -78°C. N-butyllithium
(1.6M) in hexane (36.9 mL, 59.06 mmol) was added
slowly (over a 10 min period). The mixture was

15   stirred at -78°C for 30 min (formed a gel). The
aformentioned cyclohexylacetyl chloride was added in
THF (50 mL) at -78°C. The reaction mixture was
stirred at -78°C for 30 min and then at 0°C for 1h.
The reaction was quenched by adding an aqueous

solution of NH₄Cl (16 mL). The reaction mixture was
concentrated under reduced pressure. Et₂O (300 mL)
was added. The organic phase was separated and
washed with a 10% aqueous solution of citric acid (2

5   x 200 mL), a saturated aqueous solution of NaHCO₃ (2
x 200 mL) and brine (200 mL), dried, filtered and
concentrated under reduced pressure. The residue was
purified by flash chromatography (silica gel, 40-60µ,
60 x 100 mm, 9/1 → 8/→2, hexane/EtOAc to give

10 compound **18b** as a colorless oil (11.3 g, 79% yield).
^{1}H NMR (CDCl₃) δ 4.40-4.36 (m, 1H), 4.20 (dd, J =
8.3Hz, J=9.1Hz, 1H), 4.13 (dd, J = 2.9Hz, 9.1Hz, 1H),
2.86 (dd, J = 6.4Hz, 15.7Hz, 1H), 2.65 (dd, J =
7.1Hz, 15.7Hz, 1H), 2.35-2.27 (m, 1H), 1.83-1.76 (m,

15 1H), 1.70-1.57 (m, 5H), 1.26-0.90 (m, 5H), 0.85 (d, J
= 7.0Hz, 3H), 0.81 (d, J = 6.7Hz, 3H).

Compound 18c

To a solution of compound **18b** (11.3 g, 44.68 mmol) in
20 THF (125 mL) at -78°C was added a NaHMDS solution (1M
in THF, 49.2 mL, 49.15 mmol). The reaction mixture
was stirred at -78°C for 1.5 h. A solution of *tert*-
butyl bromoacetate (8.67 mL, 53.62 mmol) in THF (25
mL) was added at -78°C. The mixture was stirred at

25 that temperature for 3h. A saturated aqueous
solution of NH₄Cl solution (33 mL) was added slowly.
The cold bath was removed and the mixture was stirred
at room temperature for 10 min. The THF was removed.
EtOAc was added (200 mL). The organic phase was

30 separated, washed serially with a saturated aqueous
solution of NaHCO₃ (200 mL), H₂O (200 mL), aqueous 1N
HCl solution (200 mL) and brine (200 mL), dried
(MgSO₄), filtered and concentrated under reduced
pressure. The residue was purified by trituration

with Et$_2$O giving compound **18c** as a white solid
(12.65g, 77% yield).

^{1}H NMR (DMSO-d$_6$) δ 4.61-4.53 (m, 3H), 4.27-4.25 (m,
1H), 2.84-2.66 (m, 2H), 2.55-2.41 (m, 1H), 1.89-1.76
5   (m, 6H), 1.58 (s, 9H), 1.35-1.31 (m, 4H), 1.14-1.04
(m, 7H).

Compound 18d

To an ice-cold solution of compound **18c** (12.2 g,
10   33.28 mmol) in a mixture of THF/H$_2$O (3/1 mixture, 495
mL/165 mL) was added H$_2$O$_2$ (30%, 15.1 mL, 133.1 mmol),
followed by a slow addition of LiOH-H$_2$O (2.79 g,
66.56 mmol). The reaction mixture was stirred at 0°C
for 1 h, then at RT overnight: The mixture was
15   cooled to 0°C and a 1.5N aqueous solution of Na$_2$SO$_3$
was added slowly to decompose excess peroxide
(monitored by KI paper). The mixture was
concentrated under reduced pressure, the residual
aqueous solution was washed with DCM (2 x 150 mL).
20   The aqueous layer was made acidic with a 10% aqueous
solution of citric acid. The mixture was extracted
with EtOAc (3 x 200 mL). The combined organic phase
were washed with brine (200 mL), dried (MgSO$_4$),
filtered and concentrated under reduced pressure.
25   Compound **18d** was obtained as a colorless oil (8.38g,
98% yield).

^{1}H NMR (CDCl$_3$) δ 2.71-2.66 (m, 1H), 2.59 (dd, J =
10.8Hz, 16.0Hz, 1H), 2.36 (dd, J = 3.8Hz, 16.0Hz,
1H), 1.78-1.57 (m, 6H), 1.41 (s, 9H), 1.30-0.98 (m,
30   5H).

Compound 18f

1) The corresponding Boc derivative of compound **18e**
(1.63 g, 2.74 mmol) was treated with HCl 4N/dioxane

(14 mL, 54.91 mmol) at RT for 1 h. The reaction
mixture was concentrated under reduced pressure. A
5% aqueous solution of Na_2CO_3 (25 mL) was added to
the residue and the resulting solution was stirred
5   vigorously for 5 min. EtOAc was added (75 mL). The
two resulting phases were separated. The organic
phase was washed with brine (50 mL), dried ($MgSO_4$),
filtered and concentrated under reduced pressure to
give **18e** which was used as such for the next step.
10 2) To the amino tripeptide in DMF (5 mL) at RT was
added compound **18d** (739 mg, 288 mmol) in DMF (5 mL),
followed by DIPEA (1.43 mL, 8.24 mmol) and TBTU (502
mg, 2.88 mmol). The reaction mixture was stirred at
RT overnight. EtOAc was added (125 mL). The organic
15 phase was separated, washed with a saturated aqueous
solution of $NaHCO_3$ (100 mL), H_2O (100 mL) and brine
(100 mL), dried ($MgSO_4$), filtered and concentrated
under reduced pressure. The residue was purified by
flash chromatography (silica gel, 40-60μ, 40 x 125mm,
20 6/4 $\rightarrow$ 5/5 hexane/EtOAc) to give the *tert*-butyl ester
compound **18f** as a white foam (1.18g, 59% yield).
^{1}H NMR ($CDCl_3$) δ 8,06 (d, J = 8.3Hz, 1H), 7.86 (d, J =
7.6Hz, 1H), 7.81 (d, J = 8.3Hz, 1H), 7.55-7.40 (m,
4H), 7.35 (s, 1H), 6.28 (d, J = 8.9Hz, 1H), 5.86-5.79
25 (m, 1H), 5.24 (dd, J = 1.6Hz, 17.2Hz, 1H), 5.17 (dd,
J = 1.3Hz, J = 10.5Hz, 1H), 4.98 (ABq, Δv=18.7Hz, J =
12.1Hz, 2H), 4.67-4.51 (m, 4H), 4.41-4.38 (m, 1H),
3.99 (dd, J = 3.8Hz, 10.8Hz, 1H), 2.64-2.59 (m, 2H),
2.42-2.38 (m, 2H), 2.10-1.95 (m, 2H), 1.68-1.53 (m,
30 9H), 1.43-1.41 (m, 1H), 1.42 (s, 9H), 1.15-1.04 (m,
4H), 0.97-0.91 (m, 8H).

Compound 18h

To the commercially available 3-[benzyl-2-
methoxycarbonylethyl)amino]propionic acid methyl
ester (**18g**) (2 g, 7.16 mmol) in MeOH (24 mL), was

5   added the palladium catalyst (Pd/C 10%, 500 mg, 25 %
w/w). The reaction mixture was stirred under a
nitrogen atmosphere (balloon) for 18 h. The mixture
was filtered through diatomaceous ester and the
filter pad was washed with MeOH (20 mL). The MeOH

10   (filtrate plus washing) was evaporated to give 1.2g
(89% yield) of compound **18h** as a pale yellow oil.
This product was used as such for the next step.

Compound 18i

15   1)   The t-butyl ester compound **18f**, (1.18 g, 1.62
mmol) was treated with 4N HCl in dioxane (8.5 mL,
32.4 mol) at RT for 6 h. The mixture was
concentrated under reduced pressure, and then co-
evaporated with benzene/Et$_2$O to give 1.04 g of the

20   corresponding acid as a beige foam (95% yield).

2)   To the latter acid (200 mg, 0.29 mmol) in DMF (1
mL) at RT was added the amine (compound **18h**, 59 mg,
0.31 mmol) in DMF (2 mL), followed by DIPEA (154 µL,

25   0.89 mmol) and TBTU (100 mg, 0.31 mmol). The
reaction mixture was stirred at RT for 72 h. EtOAc
(125 mL) was added. The organic phase was separated,
washed with a saturated aqueous solution of NaHCO$_3$
(75 mL), H$_2$O (75 mL) and brine (75 mL), dried

30   (MgSO$_4$), filtered and concentrated under reduced
pressure. The product was purified by flash
chromatography (silica gel, 40-60µ, 20 x 100 mm, 8/2
EtOAc/hexane to give compound **18i** as a yellow oil (82
mg, 33% yield).

MS (ESI) 869.3 (M+Na)$^+$, 845.4 (M–H)$^-$.

Compound 413

An aqueous 1M solution of NaOH (774 µL, 0.774 mmol)

5   was added to a solution of compound **18i** (82 mg, 0.097
mmol) in a mixture of THF/MeOH (1/1, 1 mL each). The
reaction mixture was stirred at RT for 18 h. H$_2$O was
added (15 mL). The aqueous phase was separated and
washed with DCM (3 x 15 mL). The aqueous phase was

10   made acidic (pH 3) by adding an aqueous solution of
1N HCl. The mixture was extracted with EtOAc (3 x 15
mL). The organic phase was washed with brine (25
mL), dried (MgSO$_4$), filtered and concentrated under
reduced pressure. The residue was purified by

15   preparative HPLC (5% → 53% MeCN in 60 min) to give
compound **413** as a white lyophilized solid (31 mg, 41%
yield).

MS (ESI) 779.3 (M+H)$^+$, 801.3 (M+Na)$^+$, 777.3 (M–H)$^-$

20   ^{1}H NMR (DMSO-d$_6$) δ 8.38 (S, 1H), 8.06 (d, J = 8.3Hz,
1H), 7.93 (d, J = 7.6Hz, 1H), 7.86 (d, J = 8.3Hz,
1H), 7.74 (d, J = 8.6Hz, 1H), 7.57–7.44 (m, 5H), 5.01
(d, J = 12.1Hz, 1H), 4.89 (d, J = 12.1Hz, 1H), 4.35–
4.31 (m, 2H), 4.25 (dd, J = 7.9Hz, 8.3Hz, 1H), 4.18

25   (d, J = 11.1Hz, 1H), 3.80–3.49 (m, 3H), 3.37–3.34 (m,
2H), 2.63–2.61 (m, 2H), 2.56–2.52 (m, 1H), 2.39–2.35
(m, 2H), 2.25–2.20 (m, 2H), 2.05–1.91 (m, 2H), 1.62–
1.59 (m, 1H), 1.41–1.22 (m, 5H), 0.96–0.73 (m, 16H).

Example 19

RECOMBINANT HCV NS3 PROTEASE RADIOMETRIC ASSAY

a) <u>Cloning, expression and purification of the</u>
5   <u>recombinant HCV NS3 protease type 1b</u>

Serum from an HCV-infected patient was obtained
through an external collaboration (Bernard Willems
MD, Hôpital St-Luc, Montréal, Canada and Dr. Donald
10   Murphy, Laboratoire de Santé Publique du Québec, Ste-
Anne de Bellevue, Canada). An engineered full-length
cDNA template of the HCV genome was constructed from
DNA fragments obtained by reverse transcription-PCR
(RT-PCR) of serum RNA and using specific primers
15   selected on the basis of homology between other
genotype 1b strains. From the determination of the
entire genomic sequence, a genotype 1b was assigned
to the HCV isolate according to the classification of
Simmonds et al. (J. Clin. Microbiol. (1993), <u>31</u>,
20   1493-1503.). The amino acid sequence of the non-
structural region, NS2-NS4B, was shown to be greater
than 93% identical to HCV genotype 1b (BK, JK and 483
isolates) and 88% identical to HCV genotype 1a (HCV-1
isolate). A DNA fragment encoding the polyprotein
25   precursor (NS3/NS4A/NS4B/NS5A/NS5B) was generated by
PCR and introduced into eucaryotic expression
vectors. After transient transfection, the
polyprotein processing mediated by the HCV NS3
protease was demonstrated by the presence of the
30   mature NS3 protein using Western blot analysis. The
mature NS3 protein was not observed with expression
of a polyprotein precursor containing the mutation
S1165A, which inactivates the NS3 protease,
confirming the functionality of the HCV NS3 protease.

The DNA fragment encoding the recombinant HCV NS3
protease (amino acid 1027 to 1206) was cloned in the
pET11d bacterial expression vector. The NS3 protease

5   expression in *E. coli* BL21(DE3)pLysS was induced by
incubation with 1 mM IPTG for 3 h at 22°C. A typical
fermentation (18 L) yielded approximately 100 g of
wet cell paste. The cells were resuspended in lysis
buffer (3.0 mL/g) consisting of 25 mM sodium

10   phosphate, pH 7.5, 10% glycerol (v/v), 1 mM EDTA,
0.01% NP-40 and stored at -80°C. Cells were thawed
and homogenized following the addition of 5 mM DTT.
Magnesium chloride and DNase were then added to the
homogenate at final concentrations of 20 mM and

15   20 μg/mL respectively. After a 25 min incubation at
4°C, the homogenate was sonicated and centrifuged at
15000 x *g* for 30 min at 4°C. The pH of the
supernatant was then adjusted to 6.5 using a 1M
sodium phosphate solution.

20

An additional gel filtration chromatography step was
added to the 2 step purification procedure described
in WO 95/22985 (incorporated herein by reference).
Briefly, the supernatant from the bacterial extract

25   was loaded on a SP HiTrap® column (Pharmacia)
previously equilibrated at a flow rate of 2 mL/min in
buffer A (50 mM sodium phosphate, pH 6.5, 10%
glycerol, 1 mM EDTA, 5 mM DTT, 0.01% NP-40). The
column was then washed with buffer A containing 0.15

30   M NaCl and the protease eluted by applying 10 column
volumes of a linear 0.15 to 0.3 M NaCl gradient. NS3
protease-containing fractions were pooled and diluted
to a final NaCl concentration of 0.1 M. The enzyme
was further purified on a HiTrap® Heparin column

(Pharmacia) equilibrated in buffer B (25 mM sodium phosphate, pH 7.5, 10% glycerol, 5 mM DTT, 0.01% NP-40). The sample was loaded at a flow rate of 3 mL/min. The column was then washed with buffer B

5   containing 0.15 M NaCl at a flow rate of 1.5 mL/min. Two step washes were performed in the presence of buffer B containing 0.3 or 1M NaCl. The protease was recovered in the 0.3M NaCl wash, diluted 3-fold with buffer B, reapplied on the HiTrap® Heparin column and

10   eluted with buffer B containing 0.4 M NaCl. Finally, the NS3 protease-containing fractions were applied on a Superdex 75 HiLoad® 16/60 column (Pharmacia) equilibrated in buffer B containing 0.3 M NaCl. The purity of the HCV NS3 protease obtained from the

15   pooled fractions was judged to be greater than 95% by SDS-PAGE followed by densitometry analysis.

The enzyme was stored at -80°C and was thawed on ice and diluted just prior to use.

20

b) RECOMBINANT HCV NS3 PROTEASE RADIOMETRIC ASSAY

The substrate used for the HCV NS3 protease radiometric assay, DDIVPC-SMSYTW, is cleaved between

25   the cysteine and the serine residues by the enzyme. The sequence DDIVPC-SMSYTW corresponds to the NS5A/NS5B natural cleavage site in which the cysteine residue in P2 has been substituted for a proline. The peptide substrate DDIVPC-SMSYTW and the tracer

30   biotin-DDIVPC-SMS[125I-Y]TW were incubated with the recombinant NS3 protease in the absence or in the presence of inhibitors. The separation of substrate from products was performed by adding avidin-coated agarose beads to the assay mixture followed by

filtration. The amount of SMS[^{125}I-Y]TW product found
in the filtrate (with or without inhibitor) allowed
for the calculation of the percentage of substrate
conversion and of the percentage of inhibition.

A. Reagents

Tris and Tris-HCl (UltraPure) were obtained from Life
Technologies. Glycerol (UltraPure), MES and BSA were
purchased from Sigma®. TCEP was obtained from Pierce,
DMSO from Aldrich® and NaOH from Anachemia®.

Assay buffer: 50 mM Tris-HCl, pH 7.5, 30% (w/v)
glycerol, 2% (w/v) CHAPS, 1 mg/mL BSA, 1 mM TCEP
(TCEP added just prior to use from a 1 M stock
solution in water).

Substrate: DDIVPC-SMSYTW, 25 µM final concentration
(from a 2 mM stock solution in DMSO stored at -20°C
to avoid oxidation).

Tracer: reduced mono-iodinated substrate(biotin-
DDIVPC-SMS[^{125}I-Y]TW) ($\approx$ 1 nM final concentration).

HCV NS3 protease type 1b, 25 nM final concentration
(from a stock solution in 50 mM sodium phosphate, pH
7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-
40).

B. Protocol

The assay was performed in a 96-well polypropylene
plate. Each well contained:

• 20 µL substrate/tracer in assay buffer;

100

- 10 µL ± inhibitor in 20% DMSO/assay buffer;

- 10 µL NS3 protease 1b.

Blank (no inhibitor and no enzyme) and control (no
5   inhibitor) were also prepared on the same assay
plate.

The enzymatic reaction was initiated by the addition
of the enzyme solution and the assay mixture was
10   incubated for 60 min at 23°C under gentle agitation.
Twenty (20) µL of 0.025 N NaOH were added to quench
the enzymatic reaction.

Twenty (20) µL of avidin-coated agarose beads
15   (purchased from Pierce®) were added in a Millipore®
MADP N65 filtration plate. The quenched assay mixture
was transferred to the filtration plate, and
incubated for 60 min at 23°C under gentle agitation.

20   The plates were filtered using a Millipore®
MultiScreen Vacuum Manifold Filtration apparatus, and
40 µL of the filtrate was transferred to an opaque
96-well plate containing 60 µL of scintillation fluid
per well.
25

The filtrates were counted on a Packard® TopCount
instrument using a ^{125}I-liquid protocol for 1 minute.
The %inhibition was calculated with the following
equation:
30

$$100 - [(counts_{inh} - counts_{blank})/(counts_{ctl} - counts_{blank}) \times 100]$$

A non-linear curve fit with the Hill model was
applied to the inhibition-concentration data, and the
50% effective concentration (IC_{50}) was calculated by
the use of SAS software (Statistical Software System;
5   SAS Institute, Inc., Cary, N.C.).

Example 20

**RECOMBINANT HCV NS3 PROTEASE/NS4A COFACTOR PEPTIDE
RADIOMETRIC ASSAY**

10

The enzyme was cloned, expressed and prepared
according to the protocol described in Example 19.
The enzyme was stored at -80°C, thawed on ice and
diluted just prior to use in the assay buffer
15   containing the NS4A cofactor peptide.

The substrate used for the NS3 protease/NS4A cofactor
peptide radiometric assay, DDIVPC-SMSYTW, is cleaved
between the cysteine and the serine residues by the
20   enzyme. The sequence DDIVPC-SMSYTW corresponds to the
NS5A/NS5B natural cleavage site in which the cysteine
residue in P2 has been substituted for a proline. The
peptide substrate DDIVPC-SMSYTW and the tracer
biotin-DDIVPC-SMS[^{125}I-Y]TW are incubated with the
25   recombinant NS3 protease and the NS4A peptide
cofactor KKGSVVIVGRIILSGRK (molar ratio enzyme:
cofactor 1:100) in the absence or presence of
inhibitors. The separation of substrate from products
is performed by adding avidin-coated agarose beads to
30   the assay mixture followed by filtration. The amount
of SMS[^{125}I-Y]TW product found in the filtrate allows
for the calculation of the percentage of substrate
conversion and of the percentage of inhibition.

A. Reagents

Tris and Tris-HCl (UltraPure) were obtained from Life

Technologies. Glycerol (UltraPure), MES and BSA were

5   purchased from Sigma®. TCEP was obtained from Pierce,

DMSO from Aldrich® and NaOH from Anachemia®.

Assay buffer: 50 mM Tris HCl, pH 7.5, 30% (w/v)

glycerol, 1 mg/mL BSA, 1 mM TCEP (TCEP added just

10   prior to use from a 1 M stock solution in water).

Substrate: DDIVPCSMSYTW, 25 μM final concentration

(from a 2 mM stock solution in DMSO stored at -20°C

to avoid oxidation).

15

Tracer: reduced mono iodinated substrate biotin

DDIVPC SMS[^{125}I Y]TW (~1 nM final concentration).

HCV NS3 protease type 1b, 25 nM final concentration

20   (from a stock solution in 50 mM sodium phosphate, pH

7.5, 10% glycerol, 300 mM NaCl, 5 mM DTT, 0.01% NP-

40).

NS4A Cofactor peptide: KKGSVVIVGRIILSGRK, 2.5 μM

25   final concentration (from a 2 mM stock solution in

DMSO stored at -20°C).

B. Protocol

30   The assay was performed in a 96-well polypropylene

plate. Each well contained:

• 20 μL substrate/tracer in assay buffer;

• 10 μL ± inhibitor in 20% DMSO/assay buffer;

- 10 µL NS3 protease 1b/NS4 cofactor peptide (molar ratio 1:100).

5   Blank (no inhibitor and no enzyme) and control (no inhibitor) were also prepared on the same assay plate.

The enzymatic reaction was initiated by the addition of the enzyme/NS4A peptide solution and the assay

10   mixture was incubated for 40 min at 23°C under gentle agitation. Ten (10) µL of 0.5N NaOH were added and 10 µL 1 M MES, pH 5.8 were added to quench the enzymatic reaction.

15   Twenty (20) µL of avidin-coated agarose beads (purchased from Pierce®) were added in a Millipore® MADP N65 filtration plate. The quenched assay mixture was transferred to the filtration plate, and incubated for 60 min at 23°C under gentle agitation.

20

The plates were filtered using a Millipore® MultiScreen Vacuum Manifold Filtration apparatus, and 40 µL of the filtrate was transferred in an opaque 96-well plate containing 60 µL of scintillation fluid

25   per well.

The filtrates were counted on a Packard® TopCount instrument using a ^{125}I-liquid protocol for 1 minute.

30   The value of IC_{50} was calculated in the same manner as in Example 19.

Example 21

SPECIFICITY ASSAYS

The specificity of the compounds was determined
against a variety of serine proteases: human
leukocyte elastase, porcine pancreatic elastase and
bovine pancreatic α-chymotrypsin and one cysteine
protease: human liver cathepsin B. In all cases a
96-well plate format protocol using a colorimetric p-
nitroanilide (pNA) substrate specific for each enzyme
was used. Each assay included a 1 h enzyme-inhibitor
pre-incubation at 30°C followed by addition of
substrate and hydrolysis to ≈30% conversion as
measured on a UV Thermomax® microplate reader.
Substrate concentrations were kept as low as possible
compared to K_M to reduce substrate competition.
Compound concentrations varied from 300 to 0.06 μM
depending on their potency. The final conditions for
each assay were as follows:
50mM Tris-HCl pH 8, 0.5 M Na_2SO_4, 50 mM NaCl, 0.1 mM
EDTA, 3% DMSO, 0.01% Tween-20 with;

[100 μM Succ-AAPF-pNA and 250 pM α-chymotrypsin],

[133 μM Succ-AAA-pNA and 8 nM porcine elastase], [133
μM Succ-AAV-pNA and 8 nM leukocyte elastase]; or

[100 mM $NaHPO_4$ pH 6, 0.1 mM EDTA, 3% DMSO, 1mM TCEP,
0.01% Tween-20, 30 μM Z-FR-pNA and 5 nM cathepsin B
(the stock enzyme was activated in buffer containing
20 mM TCEP before use)].

A representative example is summarized below for
porcine pancreatic elastase:

In a polystyrene flat-bottom 96-well plate were added
using a Biomek® liquid handler (Beckman):

- 40 µL of assay buffer (50 mM Tris-HCl pH 8, 50 mM
  NaCl, 0.1 mM EDTA);

5
- 20 µL of enzyme solution (50 mM Tris-/HCl pH 8, 50
  mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 40 nM
  porcine pancreatic elastase); and

- 20 µL of inhibitor solution (50 mM Tris-HCl, pH 8,
  50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 1.5 mM-

10   0.3 µM inhibitor, 15% v/v DMSO).

After 60 min pre-incubation at 30°C, 20 µL of
substrate solution (50 mM Tris-HCl, pH 8, 0.5 M
Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 665 µM Succ-AAA-pNA)
15   were added to each well and the reaction was further
incubated at 30°C for 60 min after which time the
absorbance was read on the UV Thermomax® plate
reader. Rows of wells were allocated for controls
(no inhibitor) and for blanks (no inhibitor and no
20   enzyme).

The sequential 2-fold dilutions of the inhibitor
solution were performed on a separate plate by the
liquid handler using 50 mM Tris-HCl pH 8, 50 mM NaCl,
25   0.1 mM EDTA, 0.02% Tween-20, 15% DMSO. All other
specificity assays were performed in a similar
fashion.

The percentage of inhibition was calculated using the
30   formula:

$$[1-((UV_{inh}-UV_{blank})/(UV_{ctl}-UV_{blank}))] \times 100$$

A non-linear curve fit with the Hill model was
applied to the inhibition-concentration data, and the
50% effective concentration (IC_{50}) was calculated by
the use of SAS software (Statistical Software System;
5   SAS Institute, Inc., Cary, N.C.).

Example 22
Tables of compounds

10   The following tables list IC_{50} values of compounds
representative of the invention.

The following abbreviations are used:

15   **IC_{50}**: The concentration required to obtain 50%
inhibition in the NS3 protease/NS4A cofactor peptide
radiometric assay according to example 11; the
results marked with an * indicate an IC_{50} value
obtained in the recombinant HCV NS3 protease
20   radiometric assay according to example 10;
HLE: The concentration required to obtain 50%
inhibition in the human leukocyte elastase assay;
PPE: The concentration required to obtain 50%
inhibition in the porcine pancreatic elastase assay;
25   **Other**: Figures unmarked indicate the concentration
required to obtain 50% inhibition in the bovine
pancreatic α-chymotrypsin assay; figures marked with
** indicate the concentration required to obtain 50%
inhibition in the human liver cathepsin B assay; **MS**:
30   Mass spectrometric data (MH^+ from FAB); **AAA**: amino
acid analysis data expressed in % peptide recovery;
Acca: 1-amino-cyclopropylcarboxylic acid; **Acpe**: 1-
amino-cyclopentylcarboxylic acid; **Abu**: 2-aminobutyric
acid; **Chg**: cyclohexylglycine (2-amino-2-cyclohexyl-

acetic acid); **Hyp:** 4*(R)*-hydroxyproline; **Hyp(4-Bn):**
4*(R)*-benzyloxyproline; **Pip:** pipecolic acid (i.e.
homoprolyl); **Tbg:** *tert*-butylglycine; **Ac:** acetyl; **Bn:**
benzyl; **O-Bn:** benzyloxy; **DAD:** 3-carboxypropionyl; and

5   **DAE:** 4-carboxybutyryl; **AlGly:** allylglycine (2-amino-
4-pentenoic acid); **thioxoIle:** L-thionoisoleucine; **Ph:**
phenyl; **3I-Ph:** 3-iodophenyl; **4I-Ph:** 4-iodophenyl;
2Br-Ph: 2-bromophenyl; **3Br-Ph:** 3-bromophenyl; **4Br-Ph:**
4-bromophenyl; **1-NpCH$_2$O:** naphthalen-1-ylmethoxy; **2-**

10   **NpCH$_2$O:** naphthalen-2-ylmethoxy **3,5-Br$_2$Ph:** 3,5-
dibromophenyl.

TABLE 1
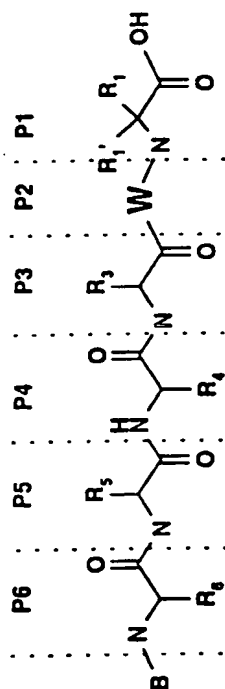
Compound	B	P6	P5	P4	P3	W	P1	IC50 (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH⁺)	AAA (%)
101	Ac	Asp	Asp	Ile	Val	Pro	Cys	46				703	113
102	Ac	Glu	Asp	Ile	Val	Pro	Cys	59				717	85.4 ± 1.6
103	DAD	---	Asp	Ile	Val	Pro	Cys	26				646	100.3 ± 1.8
104	Ac	Asp	D-Asp	Ile	Val	Pro	Cys	8.5				703	113.85 ± 4.9
105	Ac	Asp	D-Glu	Ile	Val	Pro	Cys	1.5				717	95.8 ± 0.8
106	Ac	Asp	Glu	Ile	Val	Pro	Cys	16*				717	98.8 ± 2.6
107	Ac	Asp	Val	Ile	Val	Pro	Cys	85*				687	85.9 ± 1.1
108	Ac	Asp	Tbg	Ile	Val	Pro	Cys	31				701	101.15 ± 1.65
109	Ac	Asp	Asp	Val	Val	Pro	Cys	80*				689	99.2 ± 5
110	Ac	Asp	Asp	Chg	Val	Pro	Cys	24*				729	102.95 ± 3.65
111	Ac	Asp	Asp	Tbg	Val	Pro	Cys	79				703	
112	Ac	Asp	Asp	Leu	Val	Pro	Cys	92*				703	109.7 ± 6.9
113	Ac	Asp	Asp	Ile	Ile	Pro	Cys	56*				717	72.4 ± 2.4
114	Ac	Asp	Asp	Ile	Chg	Pro	Cys	50*				743	103.65 ± 3.8
115	Ac	Asp	Asp	Ile	Val	Abu	Cys	58*				691	59.4 ± 2.85
116	Ac	Asp	Asp	Ile	Val	Leu	Cys	16*				719	95.4 ± 1.5
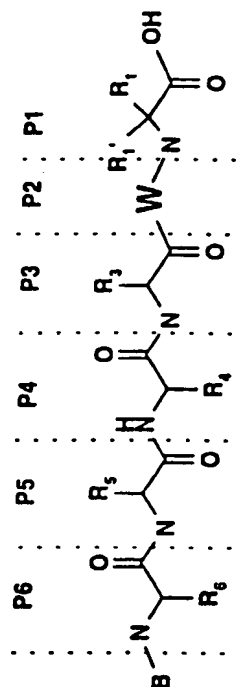
Compound	B	P6	P5	P4	P3	W	P1	IC$_{50}$ (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH$^+$)	AAA (%)
117	Ac	Asp	Asp	Ile	Val	Phe	Cys	25*				753	99.6
118	Ac	Asp	Asp	Ile	Val	Val	Cys	133*				705	96.8 ± 1
119	Ac	Asp	Asp	Ile	Val	Ile	Cys	90				719	87.0 ± 3.0
120	Ac	Asp	Asp	Ile	Val	Ala	Cys	76*				677	N.S.
121	Ac	Asp	Asp	Ile	Val	Hyp(4-Bn)	Cys	1.7				809	101
122	Ac	Asp	Asp	Ile	Val	Pro	Abu	315				685	91.0 ± 4.5
123	Ac	Asp	Asp	Ile	Val	Pro	Nva	220	>300	>300		699	107.6
124	Ac	Asp	Asp	Ile	Val	Pro	AlGly	210				697	106.3 ± 8.2
125	Ac	Asp	Asp	Ile	Val	Pro	Acpe	210				711	94.02 ± 3.19
126	Ac	Asp	Asp	Ile	Val	Pro	Acca	45				683	100.2
127	Ac	Asp	Asp	Ile	Val	Pip	Nva	605*				713	107
128	Ac	Asp	D-Glu	Ile	Val	Pro	Nva	7.4				713	100.9 ± 3.6
129	Ac	Asp	Tbg	Ile	Val	Pro	Nva	270*				697	99.8 ± 0.6
130	DAD	---	Asp	Ile.	Val	Pro	Nva	123				642	107
131	Ac	Asp	Glu	Chg	Glu	Glu	Cys	24					
132	Ac	Asp	D-Glu	Chg	Glu	Glu	Acca	36					

110
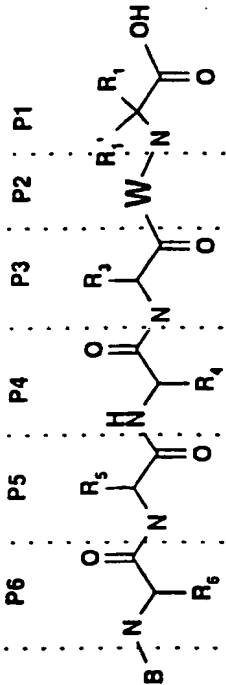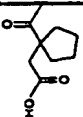
Compound	B	P6	P5	P4	P3	W	P1	IC$_{50}$ (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH$^+$)	AAA (%)
133	Ac	Asp	Glu	Chg	Val	Glu(OBn)	Acca	39					

TABLE 2
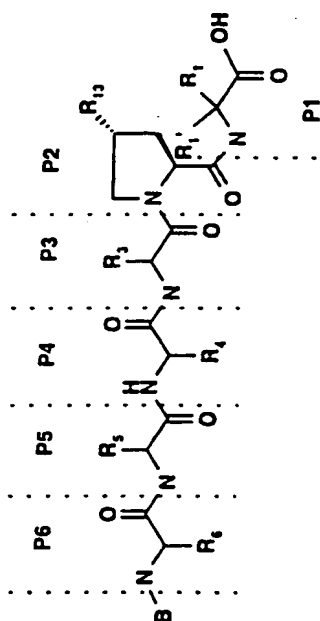
Comp.	B	P6	P5	P4	P3	R₁₃	P1	IC₅₀ (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH⁺)	AAA (%)
201	Ac	Asp	Asp	Ile	Val	O-Bn	Nva	7.2				805	107
202	Ac	Asp	D-Val	Ile	Val	O-Bn	Nva	0.93				789	103
203	Ac	Asp	D-Glu	Ile	Val	O-Bn	Nva	0.6	>300	>300	>300**	819	96.3 ± 1.7
204	Ac	Asp	Asp	Ile	Val	o-tolyl-methoxy	Nva	9.4*				819	95
205	Ac	Asp	Asp	Ile	Val	m-tolyl-methoxy	Nva	6.7*				819	98.7
206	Ac	Asp	Asp	Ile	Val	p-tolyl-methoxy	Nva	6.4*				819	101.9
207	Ac	Asp	Asp	Ile	Val	1-NpCH₂O	Nva	0.39				855	112
208	Ac	Asp	Asp	Ile	Val	2-NpCH₂O	Nva	0.71				855	104
209	Ac	Asp	Asp	Ile	Val	4-tert-butyl-phenyl)-methoxy	Nva	2.6				861	114
210	Ac	Asp	D-Glu	Chg	Val	O-Bn	Cys	0.033	>300	>300	>300	849	101.7 ± 5.4
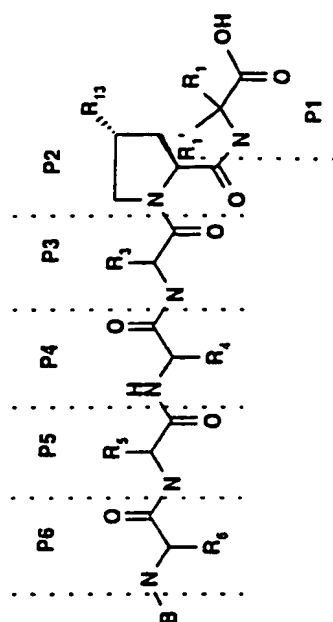
Comp.	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH+)	AAA (%)
211	Ac	Asp	D-Glu	Chg	Val	O-Bn	Nva	0.12				845	93.4 ± 2
212	Ac	Asp	D-Glu	Ile	Val	O-Bn	Acca	0.21	>300	>300		803	99.4 ± 2
213	Ac	Asp	D-Glu	Ile	Val	2-NpCH₂O	Nva	0.036				869	101.8
214	Ac	Asp	D-Glu	Chg	Val	2-NpCH₂O	Nva	0.028	>300	>300	>300 >300**	895	104.1
215	Ac	Asp	D-Glu	Chg	Val		Acca	0.014				879	---
216	Ac	Asp	Asp	.Ile	Val	Bn	Nva	60				789	100.6 ± 0.8
217	Ac	Asp	Asp	Ile	Val	Ph(CH₂)₃(	Nva	3				818	94.6 ± 3
218	Ac	Asp	D-Glu	Ile	Val	O-Bn	Nva	0.49				910	111.2
219	Ac	---	Asp	Ile	Val	1-NpCH₂O	Nva	2.3				740	95.7
220	DAD	---	---	N(Me)Ile	Val	1-NpCH₂O	Nva	31				697	---
221	DAD	---	---	Ile	Val	1-NpCH₂O	Nva	22				683	N.S.
222	DAE	---	---	Ile	Val	1-NpCH₂O	Nva	20				698	N.S.
223		---	---	Ile	Val	1-NpCH₂O	Nva	51				737	N.S.
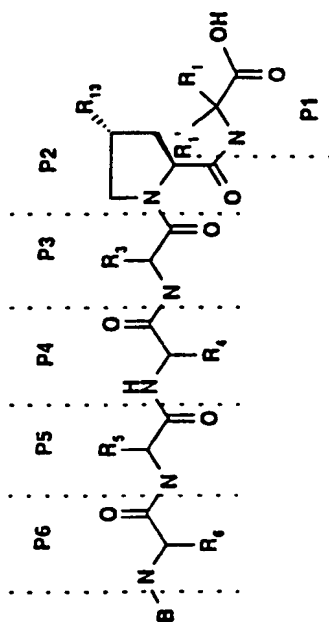
Comp.	B	P6	P5	P4	P3	R13	P1	IC50 (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH+)	AAA (%)
224	(cyclopentane-dione structure)	---	---	Ile	Val	1-NpCH2O	Nva	56				737	N.S.
225	Ac	---	---	Ile	Val	1-NpCH2O	Nva	45				929	---
226	DAE	---	---	Chg	Val	1-NpCH2O	Acca	0.76				707	---
227	Ac	---	---	Chg	Val	1-NpCH2O	Acca	3	>600			635	
228	Ac	---	---	Chg	Val	O-Bn	(cyclopropyl structure)	35	>600			613.4	
230	Ac	Asp	Asp	Ile	Val	Ph(CH2)3	Nva	3.3				818	
231	Ac	---	---	Chg	Chg	1-NpCH2O	Acca	2.6				675.4	
232	AcOCH2 -C(O)	---	---	Chg	Chg	1-NpCH2O	Acca	1.4					
233	Ac	Asp	Glu	Ile	Val	(3l-Ph)CH2O	Acca	0.14				929.2	
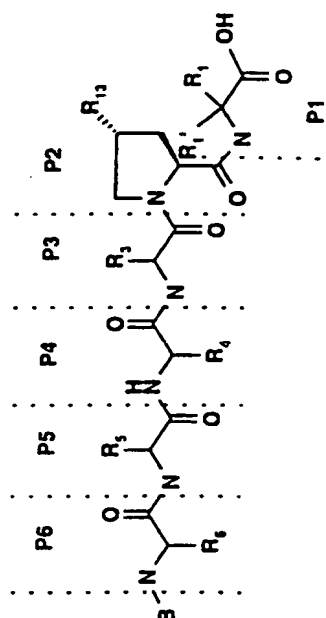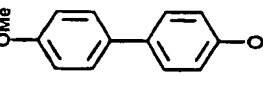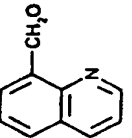
Comp.	B	P6	P5	P4	P3	R13	P1	IC50 (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH+)	AAA (%)
234	Ac	---	---	Chg	Chg	O-Bn	Acca	41					
235	Boc	---	---	Chg	Chg	1-NpCH2O	Acca	12					
236	Ac	---	Gly	thioxo-Ile	Val	1-NpCH2O	Nva	4.0				720 (M+Na)	
237	DAE	---	---	Ile	Val	1-NpCH2O	Acca	5.5				598 (M+Na)	
238	Ac	---	---	Chg	Val	(4Br-Ph)O	Acca	27					
239	Ac	---	---	Chg	Val	(2Br-Ph)O	Acca	27	195				
240	Ac	---	---	Chg	Val	(3Br-Ph)O	Acca	42					
241	Ac	---	---	Chg	Val	benzothiazolyl-S	Acca	18					
242	Ac	---	---	Chg	Val	(4Br-Ph)S	Acca	36					
243	Ac	---	---	Chg	Val	2-bromopyridinyl-O	Acca	35					
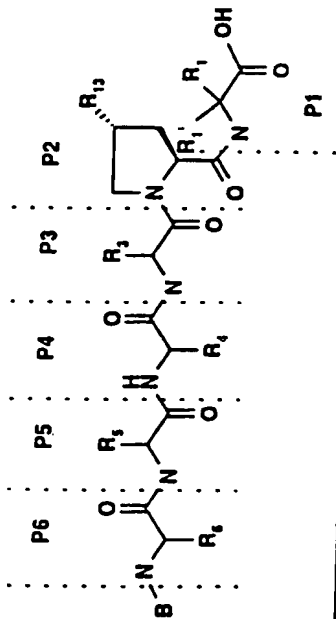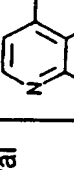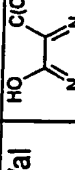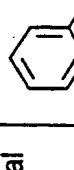
Comp.	B	P6	P5	P4	P3	R13	P1	IC$_{50}$ (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH$^+$)	AAA (%)
244	Ac	---	---	Chg	Val	(4-thio-7-CF$_3$-quinolinyl)	Acca	10					
245	Ac	---	---	Chg	Val	(4-oxy-7-CF$_3$-quinolinyl)	Acca	5.0					
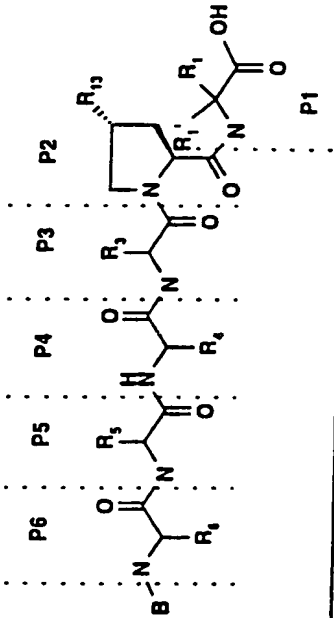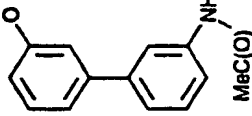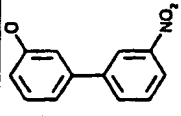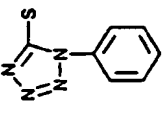
Comp.	B	P6	P5	P4	P3	R$_{13}$	P1	IC$_{50}$ (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH$^+$)	AAA (%)
246	Ac	---	---	Chg	Val	OMe (4-biphenyl-O)	Acca	33					
247	Ac	Asp	Asp	Ile	Val	Ph(CH$_2$)$_2$	Nva	10				803.6	119±1
248	Ac	---	---	Chg	Chg	(quinolin-CH$_2$O)	Acca	3.6					
249	Ac	---	---	Chg	Val	(4l-Ph)O	Acca	9.7					
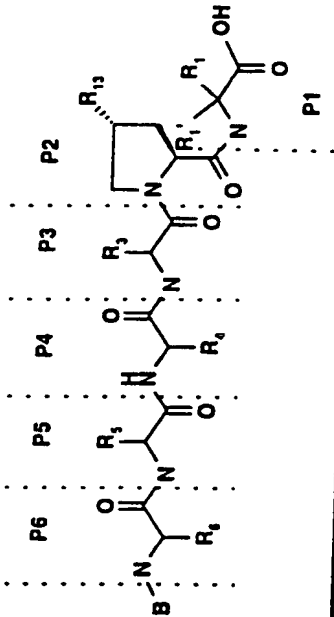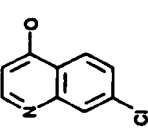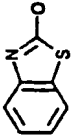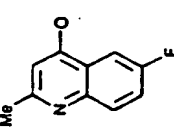
Comp.	B	P6	P5	P4	P3	R13	P1	IC$_{50}$ (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH$^+$)	AAA (%)
250	Ac	---	---	Chg	Val	[naphthol]	Acca	4.5					
251	Ac	---	---	Chg	Val	[quinoxaline diol]	Acca	13					
252	Ac	---	---	Chg	Val	1-NpCH$_2$O	Nva	20					
253	Ac	---	---	Chg	Val	[biphenyl C(O)OH]	Acca	28				651.4	91±1
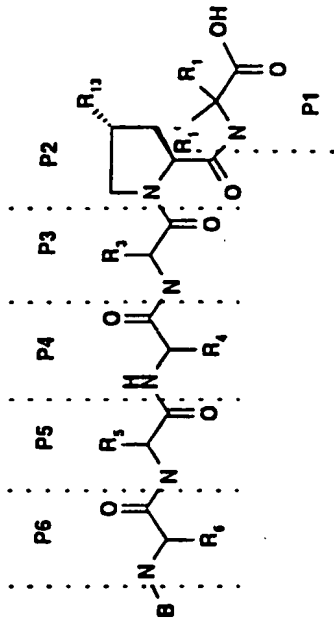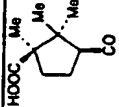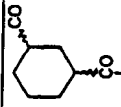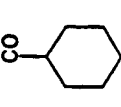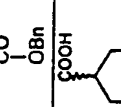
Comp.	B	P6	P5	P4	P3	R13	P1	IC50 (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH⁺)	AAA (%)
254	Ac	---	---	Chg	Val		Acca	5.1					
255	Ac	---	---	Chg	Val		Acca	4.5					
256	Ac	---	---	Chg	Val		Acca	11					
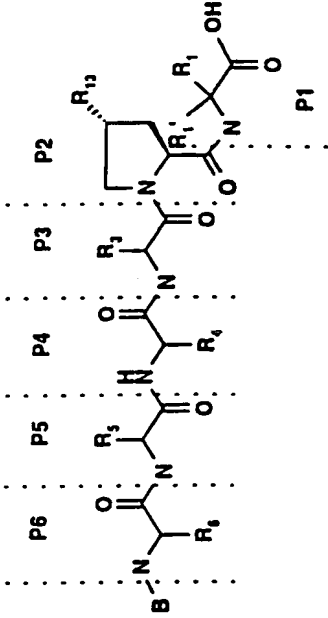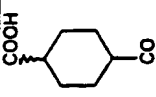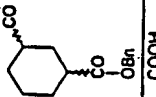
Comp.	B	P6	P5	P4	P3	R13	P1	IC50 (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH+)	AAA (%)
257	Ac	---	---	Chg	Val	[7-Cl-quinolin-4-one]	Acca	2.2	>300				
258	Ac	---	---	Chg	Val	[benzothiazol-2-one]	Acca	16					
259	Ac	---	---	Chg	Val	[6-F-2-Me-quinolin-4-one]	Acca	28					
260	Ac	Asp	D-Glu	Ile	Val	O-Bn	Cys	0.18					
261	Ac	---	---	Chg	Val	O-Bn	Cys	28					
262	Ac	---	---	Ile	Val	1-NpCH2O	Acca	40				631 (M+Na)	

SUBSTITUTE SHEET (RULE 26)
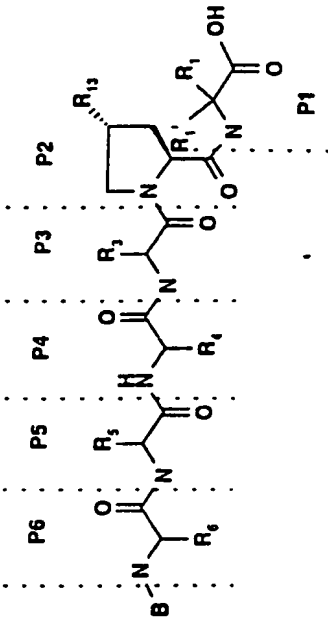
Comp.	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH⁺)	AAA (%)
263	HOOC—(Me,Me)—CO	---	---	Ile	Val	1-NpCH₂O	Acca	17				771 (M+Na)	
264	CO—⟨cyclohexane⟩—CO—OBn	---	---	Ile	Val	1-NpCH₂O	Acca	6.4				811	
265	CO—⟨cyclohexane⟩—CO—OBn	---	---	Ile	Val	1-NpCH₂O	Acca	10				811	
266	COOH—⟨cyclohexane⟩—CO	---	---	Ile	Val	1-NpCH₂O	Acca	9.7				721.4	
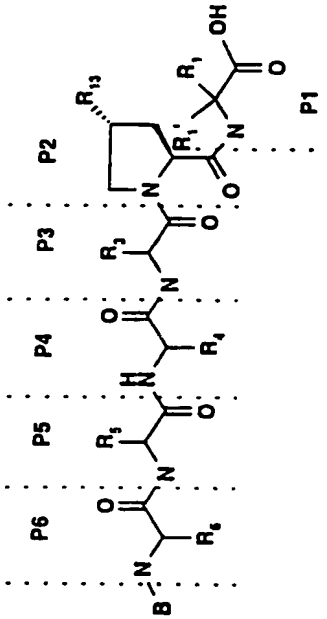
Comp.	B	P6	P5	P4	P3	R13	P1	IC50 (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH+)	AAA (%)
267	COOH–cyclohexyl–CO	---	---	Ile	Val	1-NpCH₂O	Acca	12				721.4	
268	Ac	---	---	Chg	Val	(3Br-Ph)CH₂O	Acca	24				665.1	
269	OBn / COOH–cyclohexyl–CO	---	---	Chg	Val	1-NpCH₂O	Acca	2.2				835.5 (M-H)	
270	COOH–cyclohexyl–CO	---	---	Chg	Val	1-NpCH₂O	Acca	2.0				745 (M-H)	
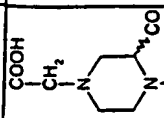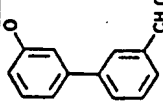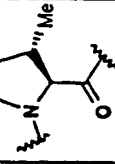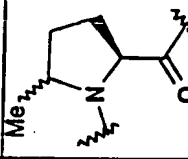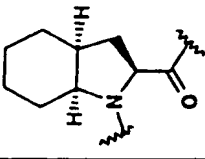
Comp.	B	P6	P5	P4	P3	R_{13}	P1	IC_{50} (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH$^+$)	AAA (%)
271	COOH–CH$_2$–N(piperidine)–CO–OBn (CO)	---	---	Chg	Val	1-NpCH$_2$O	Acca	3.8					
272	Ac	---	---	Chg	Val	(3,5-Br$_2$-Ph)CH$_2$O	Acca	27					
273	Ac	Asp	Asp	Ile	Val	H	Nva	17.5					
274	Ac	Asp	D-Val	Ile	Val	H	Cys	7.6					
275	Ac	---	---	Chg	Val	(biphenyl-CH$_2$OH)	Acca	6.2					

TABLE 3
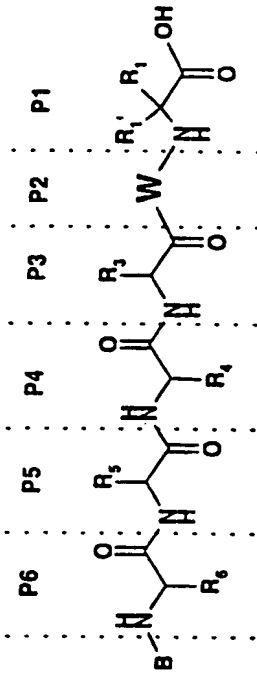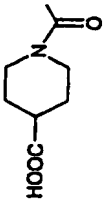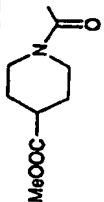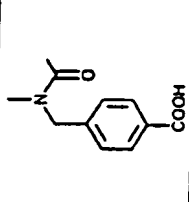
Entry #	B	P6	P5	P4	P3	W	P1	IC$_{50}$ (µM)	HLE (µM)	PPE (µM)	Other (µM)	MS (MH⁺)	AAA (%)
301	Ac	Asp	Asp	Ile	Val		Nva	98*				713	99.8
302	Ac	Asp	Asp	Ile	Val		Nva	89*				713	102
303	Ac	Asp	Asp	Ile	Val		Nva	44*				753	104.4
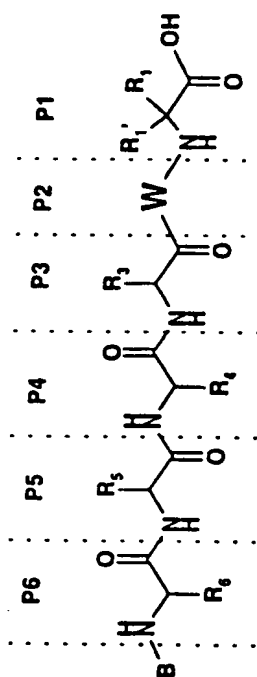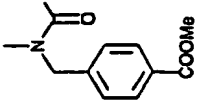
Entry #	B	P6	P5	P4	P3	W	P1	IC$_{50}$ (μM)	HLE (μM)	PPE (μM)	Other (μM)	MS (MH$^+$)	AAA (%)
304	Ac	---	---	Chg	Val	(Bn-O tetrahydroisoquinoline)	Acca	1.1					

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TABLE 4
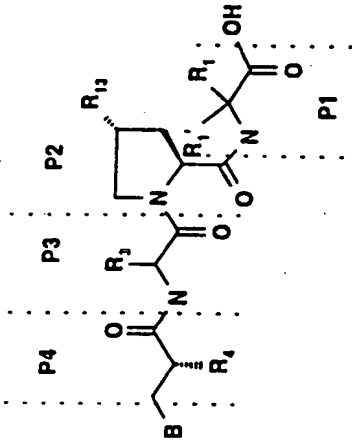
Comp.	B	P6	P5	P3	R₄	R₁₃	P1	IC₅₀ (µM)	HLE (µM)	PPE (µM)	MS (MH⁺)	AAA (%)
401	HOOC—			Val	cyclohexyl	1-NpCH₂O	Acca	7.9			747.4	
402	MeOOC—			Val	cyclohexyl	1-NpCH₂O	Acca	28			761.4	
403	COOH			Val	cyclohexyl	1-NpCH₂O	Acca	9.6			783.3	

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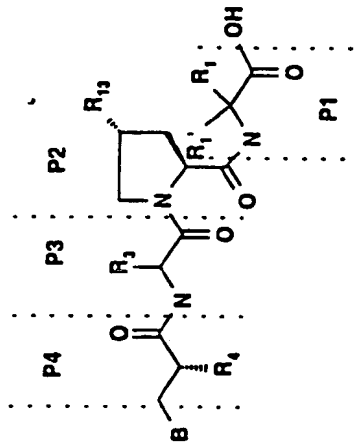
Comp.	B	P6	P5	P3	R₄	R₁₃	P1	IC₅₀ (μM)	HLE (μM)	PPE (μM)	MS (MH⁺)	AAA (%)
404	(structure with COOMe)			Val	cyclohexyl	1-NpCH₂O	Acca	13			797.3	
405	HOOC-CH₂CH₂-N(Me)C(O)-			Val	cyclohexyl	1-NpCH₂O	Acca	0.8			721.4	
406	MeOOC-CH₂-CH₂-N(Me)C(O)-			Val	cyclohexyl	1-NpCH₂O	Acca	25			735.3	

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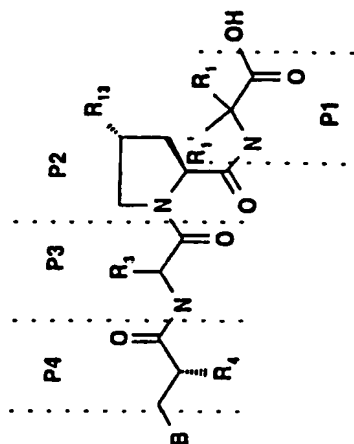
Comp.	B	P6	P5	P3	R₄	R₁₃	P1	IC₅₀ (μM)	HLE (μM)	PPE (μM)	MS (MH⁺)	AAA (%)
407	HOOC-CH₂CH₂-N(CH(Me)₂)-C(O)-			Val	cyclohexyl	1-NpCH₂O	Acca	1.5			749.3	
408	MeOOC-(CH₂)₂-N(CH(Me)₂)-C(O)-			Val	cyclohexyl	1-NpCH₂O	Acca	11			763.3	
409	HOOC-CH₂-N(CH(Me)₂)-C(O)-			Val	cyclohexyl	1-NpCH₂O	Acca	24			735.4	
410	EtOOC-CH₂-N(CH(Me)₂)-C(O)-			Val	cyclohexyl	1-NpCH₂O	Acca	32			763.4	
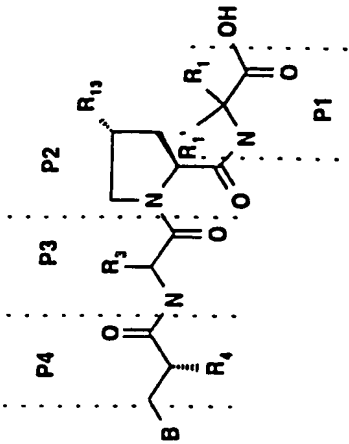
Comp.	B	P6	P5	P3	R₄	R₁₃	P1	IC₅₀ (μM)	HLE (μM)	PPE (μM)	MS (MH⁺)	AAA (%)
411	HOOC-(CH₂)₃-N(CH(Me)₂-C(O)			Val	cyclohexyl	1-NpCH₂O	Acca	7.4			763.4	
412	[HOOC-CH₂]₂-NC(O)-			Val	cyclohexyl	1-NpCH₂O	Acca	0.8			751.3	
413	[HOOC-(CH₂)₂]₂-NC(O)-			Val	cyclohexyl	1-NpCH₂O	Acca	0.12			779.3	
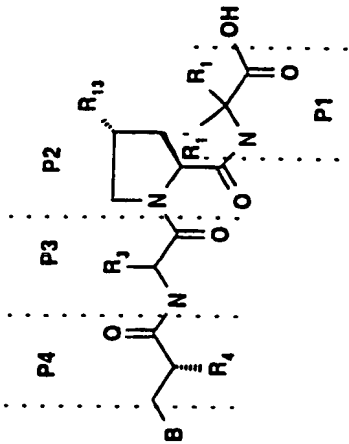
Comp.	B	P6	P5	P3	R4	R13	P1	IC50 (μM)	HLE (μM)	PPE (μM)	MS (MH+)	AAA (%)
414				Val	cyclohexyl	1-NpCH2O	Acca	0.78			761.3	
415				Val	cyclohexyl	1-NpCH2O	Acca	0.89			803.2	
416				Val	cyclohexyl	1-NpCH2O	Acca	0.41			791.1	
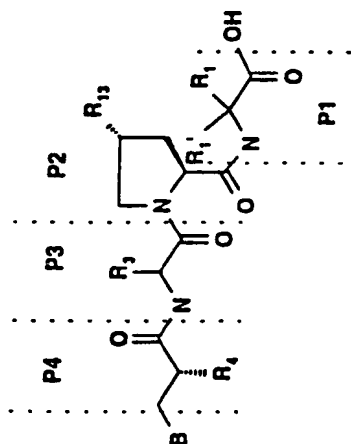
Comp.	B	P6	P5	P3	R4	R13	P1	IC50 (μM)	HLE (μM)	PPE (μM)	MS (MH+)	AAA (%)
417				Val	cyclohexyl	1-NpCH2O	Acca	0.45			763.2	
418				Val	cyclohexyl	1-NpCH2O	Acca	0.63			797.3	
419					cyclohexyl	1-NpCH2O	Acca	1.4			775.6 (M-H)-	
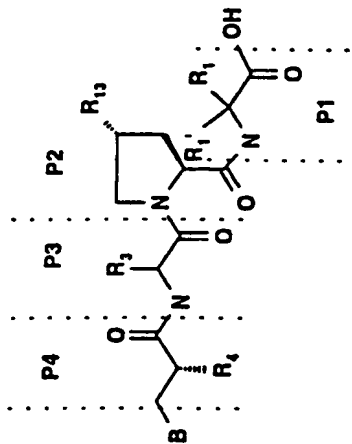
Comp.	B	P6	P5	P3	R₄	R₁₃	P1	IC₅₀ (µM)	HLE (µM)	PPE (µM)	MS (MH⁺)	AAA (%)
420	(HO₂C...N-acetyl, Ph₂CHCH₂)			Val	cyclohexyl	1-NpCH₂O	Acca	0.52			925.6 (MK)⁺	
421	(HO₂C...N-acetyl, thiophene)			Val	cyclohexyl	1-NpCH₂O	Acca	1.7			841.5 (MK)⁺	
422	(HO₂C...N-acetyl, NMe₂)			Val	cyclohexyl	1-NpCH₂O	Acca	4.0			778.4	

SUBSTITUTE SHEET (RULE 26)
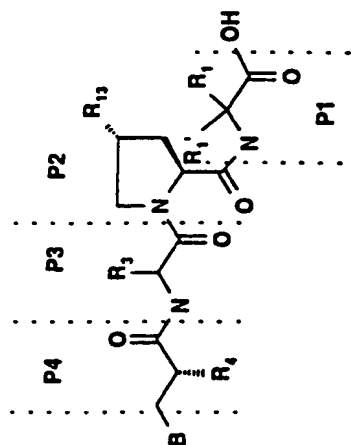
Comp.	B	P6	P5	P3	R₄	R₁₃	P1	IC₅₀ (μM)	HLE (μM)	PPE (μM)	MS (MH⁺)	AAA (%)
423				Val	cyclohexyl	1-NpCH₂O	Acca	7.9			726.3	

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What is claimed is:

1.   A compound of formula (I):
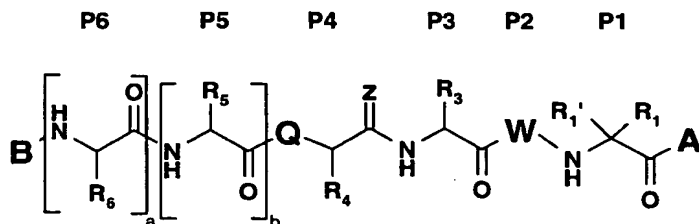
(I)

wherein **Q** is CH_2 or N-**Y**, wherein **Y** is H or C_{1-6} alkyl;

a) when **Q** is CH_2, **a** is 0, **b** is 0, and **B** is an amide
derivative of formula $R_{11a}N(R_{11b})-C(O)-$ wherein R_{11a} is
H; C_{1-10} alkyl optionally substituted with carboxyl or
di(loweralkyl) amino; C_{3-7} cycloalkyl; C_6 aryl; C_{7-10}
alkylaryl; (C_{3-7} cycloalkyl)-(C_{1-6} alkyl); heterocycle-
C_{1-6} alkyl;

and R_{11b} is C_{1-6} alkyl substituted with carboxyl, (C_{1-6}
alkoxy)carbonyl or phenylmethoxycarbonyl; or C_{7-16}
aralkyl substituted on the aromatic portion with
carboxyl, (C_{1-6} alkoxy)carbonyl,
phenylmethoxycarbonyl, or heterocycle-C_{1-6} alkyl;
or R_{11a} and R_{11b} are joined to form a 3 to 7-membered
nitrogen-containing ring optionally substituted with
carboxyl or (C_{1-6} alkoxy) carbonyl;

or

b) when **Q** is N-**Y**; **a** is 0 or 1, **b** is 0 or 1, and **B** is
an acyl derivative of formula $R_{11}-C(O)-$wherein R_{11} is
(i) C_{1-10} alkyl optionally substituted with carboxyl,
C_{1-6} alkanoyloxy (e.g. $AcOCH_2$) or C_{1-6} alkoxy (e.g.
Boc); (ii) C_{3-7} cycloalkyl optionally substituted with
carboxyl, (C_{1-6} alkoxy)carbonyl or

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phenylmethoxycarbonyl; (iii) C_{3-7} cycloalkyl
substituted with carboxyl and one to three C_{1-6} alkyl
substituents (iv) C_{4-10} (alkylcycloalkyl) optionally
substituted on the cycloalkyl portion with carboxy,
(C$_{1-6}$ alkoxy)carbonyl or phenylmethyoxycarbonyl; (v)

HOOC-(C$_{1-6}$alkyl)-N⟨   ⟩NCOO-(aryl or C$_{1-6}$ alkylaryl) ,
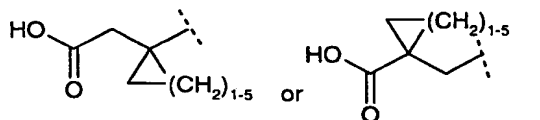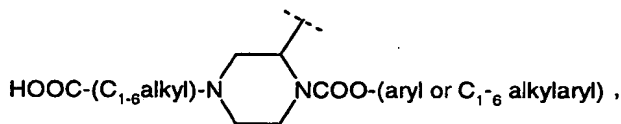
(v) C_6 or C_{10} aryl or C_{7-16} aralkyl optionally
substituted with C_{1-6} alkyl;

R$_6$, when present, is C_{1-6} alkyl substituted with
carboxyl; and

R$_5$, when present, is C_{1-6} alkyl optionally substituted
with carboxyl;

or

c) when **Q** is either CH_2 or **N-Y**;

R$_4$ is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}
(alkylcycloalkyl);

Z is oxo or thioxo;

R$_3$ is C_{1-10} alkyl optionally substituted with
carboxyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

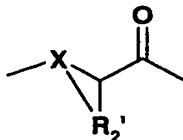**W** is a group of formula II:

Formula II

wherein **R₂** is C_{1-10} alkyl or C_{3-10} cycloalkyl optionally substituted with carboxyl; C_6 or C_{10} aryl or C_{7-16} aralkyl; or

W is a group of formula II':

Formula II'

wherein **X** is CH or N; and

R₂' is divalent C_{3-4} alkylene which together with **X** and the carbon atom to which **X** and **R₂'** are attached form a 5- or 6-membered ring, said ring optionally substituted with OH; SH; NH_2; carboxyl; **R₁₂**; OR_{12}, $C(O)OR_{12}$, SR_{12}, NHR_{12} or $NR_{12}R_{12}'$ wherein **R₁₂** and **R₁₂'** are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl, said alkyl or alkenyl optionally substituted with NH_2, OH, SH, halo, or carboxyl; said alkyl or alkenyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N; or

R₁₂ and **R₁₂'** are independently C_6 or C_{10} aryl or C_{7-16} aralkyl optionally substituted with C_{1-6} alkyl, CF_3, NH_2, OH, SH, halo, carboxyl, C_{1-6} alkyl substituted with carboxyl, phenyl optionally substituted with C_{1-6} alkyl, C_{1-6} alkoxy, halo, acetylamido or nitro; said aryl or aralkyl optionally containing at least one heteroatom selected independently from the group consisting of: O, S, and N;

said cyclic alkyl, cyclic alkenyl, aryl or aralkyl being optionally fused with a second 5-,

6-, or 7-membered ring to form a cyclic system
or heterocyclic system, said second ring being
optionally substituted with NH_2, OH, SH, halo,
carboxyl or carboxy(lower)alkyl; said second

5   ring optionally containing at least one
heteroatom selected independently from the group
consisting of: O, S, and N;

or **X** is CH or N; and **$R_{2'}$** is a divalent C_{3-4} alkylene
which together with **X** and the carbon atom to which **X**

10   and **$R_{2'}$** are attached form a 5- or 6-membered ring
which in turn is fused with a second 5-, 6- or 7-
membered ring to form a cyclic system wherein the
second ring is substituted with $OR_{12''}$ wherein **$R_{12''}$** is
C_{7-16} aralkyl;

15   **R_1'** is hydrogen, and **R_1** is C_{1-6} alkyl optionally
substituted with thiol or halo; or **R_1** is C_{2-6} alkenyl;
or

R_1' and **R_1** together form a 3- to 6-membered ring
optionally substituted with C_{1-6} alkyl; and

20   **A** is hydroxy or a pharmaceutically acceptable salt or
ester thereof.

2.   A compound of formula (Ia):

(Ia)
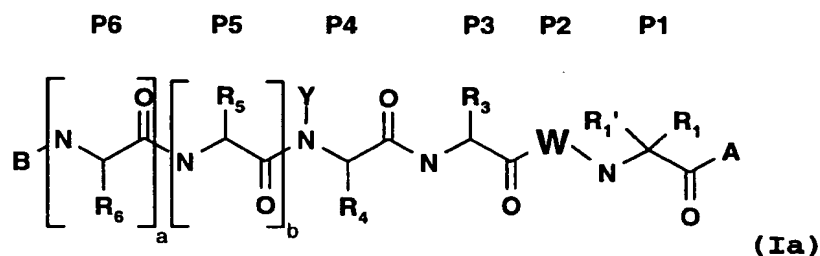
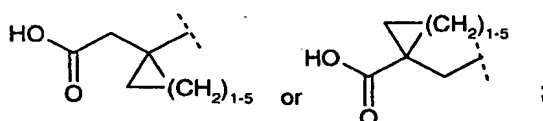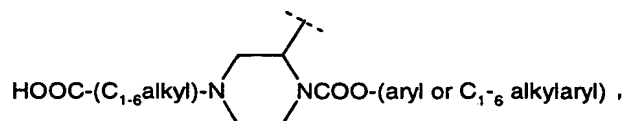25   wherein **Y** is H or C_{1-6} alkyl;

a is 0 or 1;

b is 0 or 1;

B is an acyl derivative of formula R_{11}-C(O)-wherein
R_{11} is (i) C_{1-10} alkyl optionally substituted with

carboxyl, C_{1-6} alkanoyloxy or C_{1-6} alkoxy; (ii) C_{3-7}
cycloalkyl optionally substituted with carboxyl, (C_{1-6}
alkoxy)carbonyl or phenylmethoxycarbonyl; (iii) C_{3-7}
cycloalkyl substituted with carboxyl and one to three
C_{1-6} alkyl substituents (iv) C_{4-10} (alkylcycloalkyl)
optionally substituted on the cycloalkyl portion with
carboxy, (C_{1-6} alkoxy)carbonyl or
phenylmethyoxycarbonyl; (v)

HOOC-(C_{1-6}alkyl)-N⟨ ⟩NCOO-(aryl or C_{1-6} alkylaryl) ,

HO—...—$(CH_2)_{1-5}$ or HO—...—$(CH_2)_{1-5}$;

(v) C_6 or C_{10} aryl or C_{7-16} aralkyl optionally
substituted with C_{1-6} alkyl;

R₆, when present, is C_{1-6} alkyl substituted with
carboxyl;

R₅, when present, is C_{1-6} alkyl optionally substituted
with carboxyl; and

R₄ is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}
(alkylcycloalkyl);

R₃, **W**, **R₁**, **R₁'** and **A** are as defined in claim 1.

3.   A compound of formula Ia according to claim 2,
wherein **B** is an acyl derivative of formula $R_{11}C(O)-$
wherein **R₁₁** is:
C_{1-6} alkyl optionally substituted with carboxyl, C_{1-6}
alkanoyloxy or C_{1-6} alkoxy;
C_{3-7} cycloalkyl optionally substituted with carboxyl,
MeOC(O), EtOC(O) or BnOC(O);
3-carboxypropionyl (DAD) or 4-carboxybutyryl (DAE);
or

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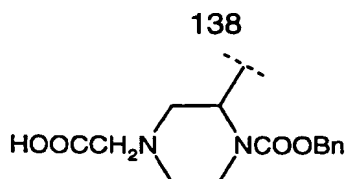
4.   A compound of formula Ia according to claim 3, wherein **B** is acetyl, 3-carboxypropionyl, 4-carboxylbutyryl, AcOCH$_2$C(O), Me$_3$COC(O),
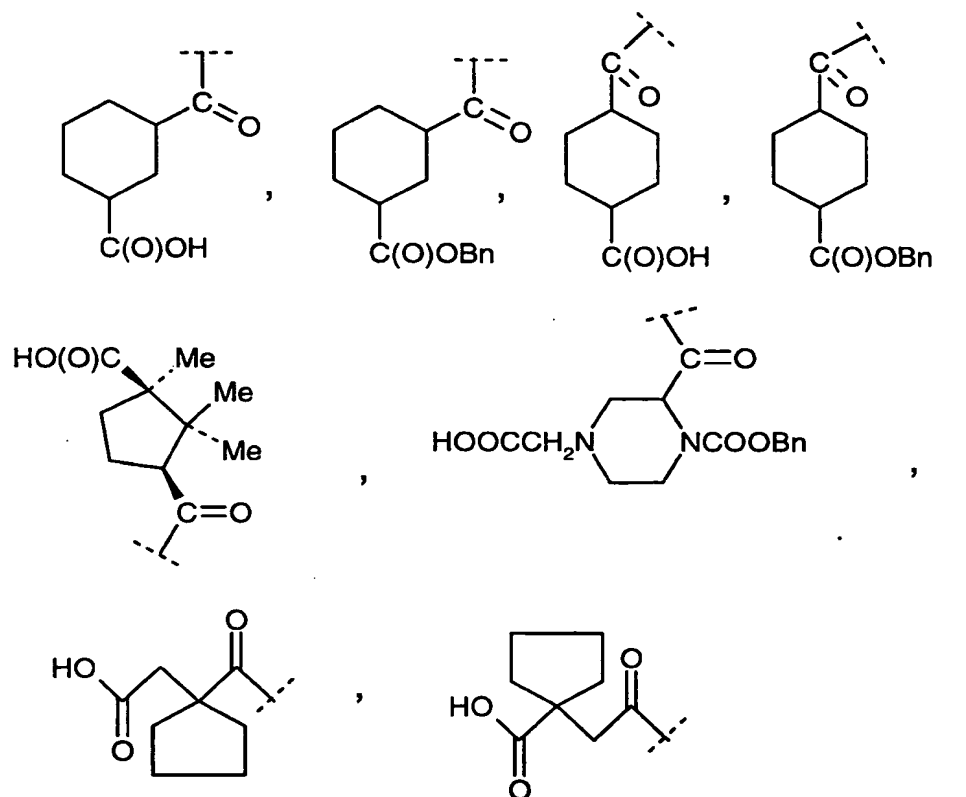
5.   A compound of formula Ia according to claim 4, wherein **B** is acetyl, 3-carboxypropionyl (DAD), 4-carboxybutyryl (DAE), AcOCH$_2$C(O),
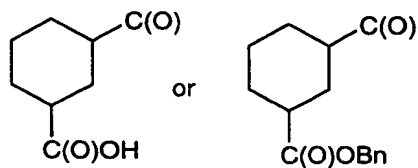
6.   A compound of formula Ia according to claim 5,
wherein, **B** is acetyl.

7.   A compound of formula Ia according to claim 2,
wherein R_6, when present, is the side chain of Asp or
Glu.

8.   A compound of formula Ia according to claim 7,
wherein R_6, when present, is the side chain of Asp.

9.   A compound of formula Ia according to claim 2,
wherein R_5, when present, is the side chain of an
amino acid selected from the group consisting of D-
Asp, Asp, D-Glu, Glu, D-Val, Val, D-Tbg and Tbg.

10.   A compound of formula Ia according to claim 9,
wherein R_5, when present, is the side chain of D-Asp,
D-Val or D-Glu.

11.   A compound of formula Ia according to claim 10,
wherein R_5, when present, is the side chain of D-Glu.

12.   A compound of formula (Ib):
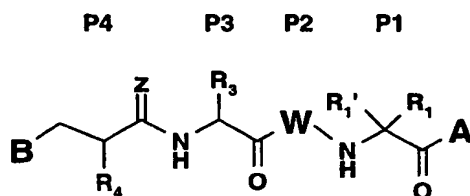
P4   P3   P2   P1

(Ib)

wherein **B** is an amide of formula $R_{11a}N(R_{11b})C(O)-$
wherein R_{11a} is C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{3-7}
(alkylcylcoalkyl) optionally substituted with
carboxy, C_{1-3} carboxyalkyl, C_6 aryl, C_{7-10} arylalkyl,
2-tetrahydrofuranylmethyl, or 2-thiazolidylmethyl;

and R_{11b} is C_{1-6} alkyl substituted with carboxyl.

13.   A compound of formula (Ib) according to claim
12, wherein R_{11a} is cyclopropylmethyl, isopropyl,
5   carboxyethyl, benzylmethyl, benzyl, or 2-
tetrahydrofuranylmethyl.

14.   A compound of formula (Ib) according to claim
13, wherein R_{11b} is C_{1-4} alkyl substituted with
10   carboxyl.

15.   A compound of formula (Ib) according to claim
14, wherein R_{11b} is ethyl carboxyl.

15   16.   A compound of formula I according to claim 1,
wherein R_4 is selected from the group consisting of:
isopropyl, cyclopropyl, tert-butyl, 1-methylpropyl,
or 2-methylpropyl.

20   17.   A compound of formula I according to claim 16,
wherein R_4 is cyclopropyl or 1-methylpropyl.

18.   A compound of formula Ia according to claim 17,
wherein R_4 is cyclopropyl.
25

19.   A compound of formula I according to claim 1,
wherein Z is oxo.

20.   A compound of formula I according to claim 1,
30   wherein R_3 is the side chain of Ile, allo-Ile, Chg,
Cha, Val, Tbg or Glu.

21.   A compound of formula I according to claim 20,
wherein R_3 is the side chain of Val, Tbg or Chg.

22. A compound of formula I according to claim 21,
wherein **R₃** is the side chain of Val.

5   23. A compound of formula I according to claim 1,
wherein **W** is a group of formula II wherein **R₂** is C_{1-6}
alkyl; C_{1-6} alkyl substituted with carboxyl, C_{1-6}
alkoxycarbonyl, benzyloxycarbonyl or
benzylaminocarbonyl; or benzyl.

10

24. A compound of formula I according to claim 23,
wherein **W** is a group of formula II wherein **R₂** is the
side chain of Abu, Leu, Phe, Cha, Val, Ala, Asp, Glu,
Glu(OBn) or Glu (NHBn).

15

25. A compound of formula I according to claim 24,
wherein **R₂** is the side chain of Asp, aminobutyric
acid (Abu) or Val.

20   26. A compound of claim I according to claim 1,
wherein **W** is a group of formula III'
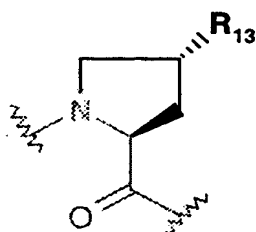
Formula III'

wherein **R₁₃** is OH; SH; NH₂; carboxyl; **R₁₂**; OR₁₂, SR₁₂,
25   NHR₁₂ or NR₁₂R₁₂' wherein **R₁₂** and **R₁₂'** are
independently:
          cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or
          cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl,
          said alkyl or alkenyl optionally substituted
30   with NH₂, OH, SH, halo, or carboxyl; said alkyl

or alkenyl optionally containing at least one
heteroatom independently selected from the group
consisting of: O, S, and N; or

R_{12} and R_{12}' are independently C_6 or C_{10} aryl or

5   C_{7-16} aralkyl optionally substituted with C_{1-6}
alkyl, NH_2, OH, SH, halo, carboxyl or
carboxy(lower)alkyl; said aryl or aralkyl
optionally containing at least one heteroatom
independently selected from the group consisting

10   of: O, S, and N;
said cyclic alkyl, cyclic alkenyl, aryl or aralkyl
being optionally fused with a second 5-, 6-, or 7-
membered ring to form a cyclic system or heterocyclic
system, said second ring being optionally substituted

15   with NH_2, OH, SH, halo, carboxyl or
carboxy(lower)alkyl; said second ring optionally
containing at least one heteroatom independently
selected from the group consisting of: O, S, and N.

20   27. A compound of claim I according to claim 26,
wherein R_{13} is OR_{12} or SR_{12} wherein R_{12} is a C_6 or C_{10}
aryl or C_{7-16} aralkyl, said first aryl or aralkyl
optionally substituted with C_{1-6} alkyl, C_{3-7}
cycloalkyl, NH_2, OH, SH, halo, C_{1-6} alkoxy, carboxyl,

25   carboxy(lower)alkyl, or a second aryl or aralkyl;
said first and second aryl or aralkyl optionally
containing at least one heteroatom selected
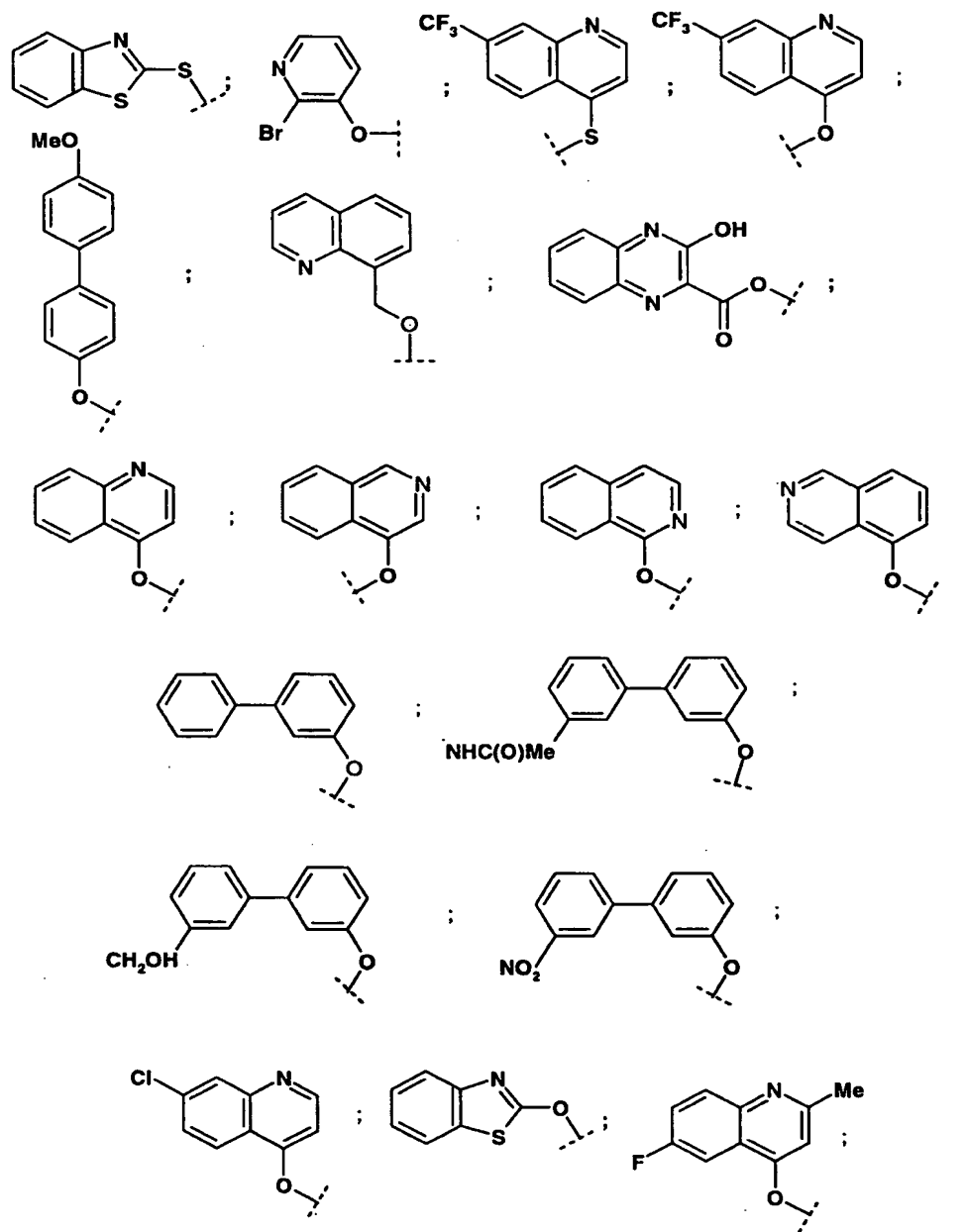independently from the group consisting of: O, S, and
N.

30

28. A compound according to claim 27, wherein R_{13} is
Bn; $PhCH_2CH_2$; $PhCH_2CH_2CH_2$; O-Bn; o-tolylmethoxy; m-
tolylmethoxy; p-tolylmethoxy; 1-naphtyloxy; 2-
naphtyloxy; 1-naphthalenylmethoxy; 2-

naphthalenylmethoxy; (4-tert-butyl)methoxy; (3I-
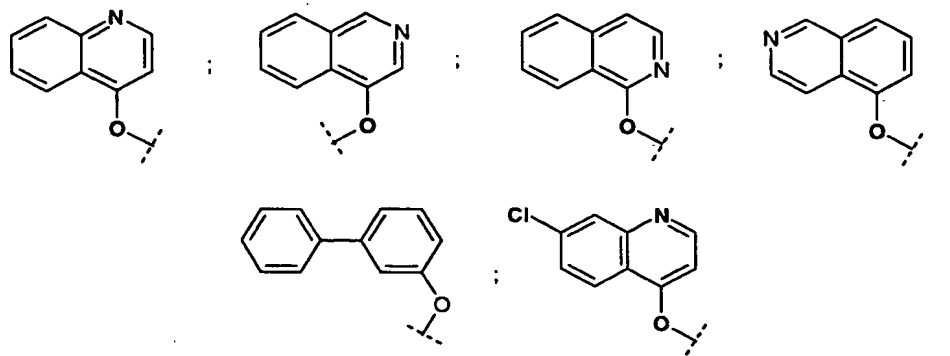Ph)CH₂O; (4Br-Ph)O; (2Br-Ph)O; (3Br-Ph)O; (4I-Ph)O;
(3Br-Ph)CH₂O; (3,5-Br₂-Ph)CH₂O;

29. A compound according to claim 28, wherein R₁₃ is
O-Bn; PhCH₂CH₂CH₂; 1-naphtyloxy; 2-naphtyloxy; 1-

naphthalenylmethoxy; 2-naphthalenylmethoxy;

5

30.   A compound of formula I according to claim 1,
wherein $R_{1'}$ is hydrogen and R_1 is C_{1-6} alkyl optionally
substituted with thiol.

10   31.   A compound of formula I according to claim 30,
wherein $R_{1'}$ is the side chain of the amino acid
selected from the group consisting of: cysteine
(Cys), aminobutyric acid (Abu), norvaline (Nva), or
allylglycine (AlGly).

15

32.   A compound of formula I according to claim 31,
wherein R_1' is H and R_1 is propyl.

33.   A compound of formula I according to claim 1,
20   wherein $R_{1'}$ and R_1 together form a 3- to 6-membered
ring, said ring being optionally substituted with
ethyl.

34.   A compound of formula I according to claim 33,
25   wherein $R_{1'}$ and R_1 together form a cyclopropyl,
cyclobutyl, cyclopentyl or cyclohexyl ring.

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35. A compound of formula I according to claim 34, wherein R_1 and $R_{1'}$ together form a cyclopropyl ring optionally substituted with C1-6 alkyl.

5   36. A compound of formula I according to claim 1, wherein

a) Q is CH_2, a is 0, b is 0, and B is an amide of formula $R_{11a}N(R_{11b})-C(O)-$ wherein R_{11a} is C_{1-6} alkyl optionally substituted with carboxyl, C_{3-6} cycloalkyl,

10   C_{3-7} (alkylcycloalkyl) optionally substituted with carboxy, $(C_{1-3}$ alkoxy)carbonyl, phenyl, C_{7-10} arylalkyl, 2-tetrahydrofuranylmethyl, or 2-thienylmethyl;

and R_{11b} is $(C_{0-2}$ alkyl)phenyl optionally substituted

15   with carboxyl or $(C_{1-4}$ alkoxy)carbonyl; or C_{1-6} alkyl substituted with carboxyl or $(C_{1-4}$ alkoxy)carbonyl; or R_{11a} and R_{11b} are joined to form a piperidine ring optionally substituted with carboxyl or $(C_{1-6}$ alkoxy)carbonyl;

20   or

b) Q is N-Y, wherein Y is H or C_{1-6} alkyl; a is 0 or 1, b is 0 or 1, and B is an acyl derivative of formula $R_{11}-C(O)-$ wherein R_{11} is (i) C_{1-6} alkyl, C_{1-6} alkyl substituted with carboxyl, MeC(O)O-, MeO-, EtO-

25   , MeCH₂CH₂O- or Me₃C-O-; (ii) cyclopentyl or cyclohexyl optionally substituted with carboxyl; (iv) C_{4-10} (alkylcycloalkyl) optionally substituted on the cycloalkyl portion with carboxyl;

(v)

HOOCCH₂N⟨ ⟩NCOOBn
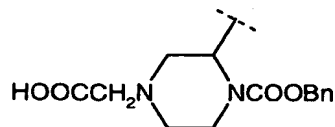
30

(vi) phenyl, benzyl or phenylethyl;

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R_6, when present, is CH_2COOH or CH_2CH_2COOH,

R_5, when present, is C_{1-6} alkyl or CH_2COOH or

CH_2CH_2COOH; or

5   c) when Q is either CH_2 or $N-Y$,

R_4 is C_{1-6} alkyl, C_{3-7} cycloalkyl or C_{4-10}

(alkylcycloalkyl);

Z is oxo or thio;

R_3 is C_{1-6} alkyl; C_{3-7} cycloalkyl or C_{4-10}

10   (alkylcycloalkyl);

W is a group of formula II wherein R_2 is C_{1-10} alkyl,

C_{3-10} cycloalkyl, C_{7-11} aralkyl; CH_2COOH or CH_2CH_2COOH;

or W is a group of formula II' wherein X is N or CH

and $R_{2'}$ is the divalent radical $-CH_2CH_2CH_2-$ or $-$

15     $CH_2CH_2CH_2CH_2-$ which together with X and the carbon

atom to which X and $R_{2'}$ are attached form a 5- or 6-

membered ring, said ring optionally substituted with

OR_{12}, $C(O)OR_{12}$, SR_{12}, NHR_{12} or $NR_{12}R_{12'}$ wherein R_{12} and

$R_{12'}$ are independently:

20   cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or

cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl,

said alkyl or alkenyl optionally substituted

with NH_2, OH, SH, halo, or carboxyl; said alkyl

or alkenyl optionally containing at least one

25   heteroatom independently selected from the grop

consisting of: O, S, and N; or R_{12} and $R_{12'}$ are

independently C_6 or C_{10} aryl or C_{7-16} aralkyl

optionally substituted with C_{1-6} alkyl, CF_3, NH_2,

OH, SH, halo, carboxyl, C_{1-6} alkyl substituted

30   with carboxyl, or phenyl optionally substituted

with C_{1-6} alkyl, C_{1-6} alkoxy or halo; said aryl or

aralkyl optionally containing at least one

heteroatom independently selected from the group

consisting of: O, S, and N; said cyclic alkyl,

cyclic alkenyl, aryl or aralkyl being optionally
fused with a second 5-, 6-, or 7-membered ring
to form a cyclic system or heterocyclic system,
said second ring being optionally substituted

5   with NH_2, OH, SH, halo, carboxyl or C_{1-6} alkyl
substituted with carboxyl; said second ring
optionally containing at least one heteroatom
independently selected from the group consisting
of: O, S, and N; or **X** is N; and **R$_2$,** is -

10       $CH_2CH_2CH_2-$ or $-CH_2CH_2CH_2CH-$ which together with **X**
and the carbon atom to which **X** and **R$_2$,** are
attached form a 5- or 6-membered ring, which in
turn is fused to a phenyl to form a cyclic
system wherein the phenyl ring is substituted

15   with O**R$_{12}$,,** wherein **R$_{12}$,,** is phenylmethyl or
phenylethyl;

R$_1$, is hydrogen and **R$_1$** is methyl, thiomethyl, 1-
methylethyl, propyl, 1-methylpropyl, 2-
(methylthio)ethyl or 2-propylene; or **R$_1$,** and **R$_1$**

20   together with the carbon atom to which they are
attached form a cyclopropyl which may optionally be
substituted with ethyl; and
A is hydroxy or a pharmaceutically acceptable salt
thereof; C_{1-6} alkoxy, or (aryl C_{1-6}-alkoxy).

25

37.   A compound of formula Ia according to claim 2,
B is an acyl derivative of formula **R$_{11}$**-C(O)- wherein
R$_{11}$ is C_{1-6} alkoxy, C_{1-10} alkyl optionally substituted
with carboxyl; C_{3-7} cycloalkyl optionally substituted

30   with carboxyl or benzylcarboxy; or

HOOCCH$_2$N   NCOOBn   ;
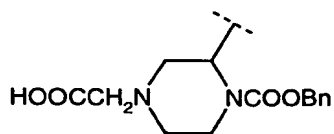
R_6 is absent;

R_5 is absent;

R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);

5   R_3 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10} (alkylcycloalkyl);
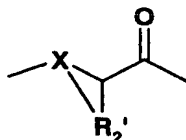
W is a group of formula II:
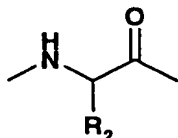
Formula II

10   wherein R_2 is C_{1-6} alkyl; C_{3-6} cycloalkyl; C_{1-6} alkyl substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-11} aralkyl;

or

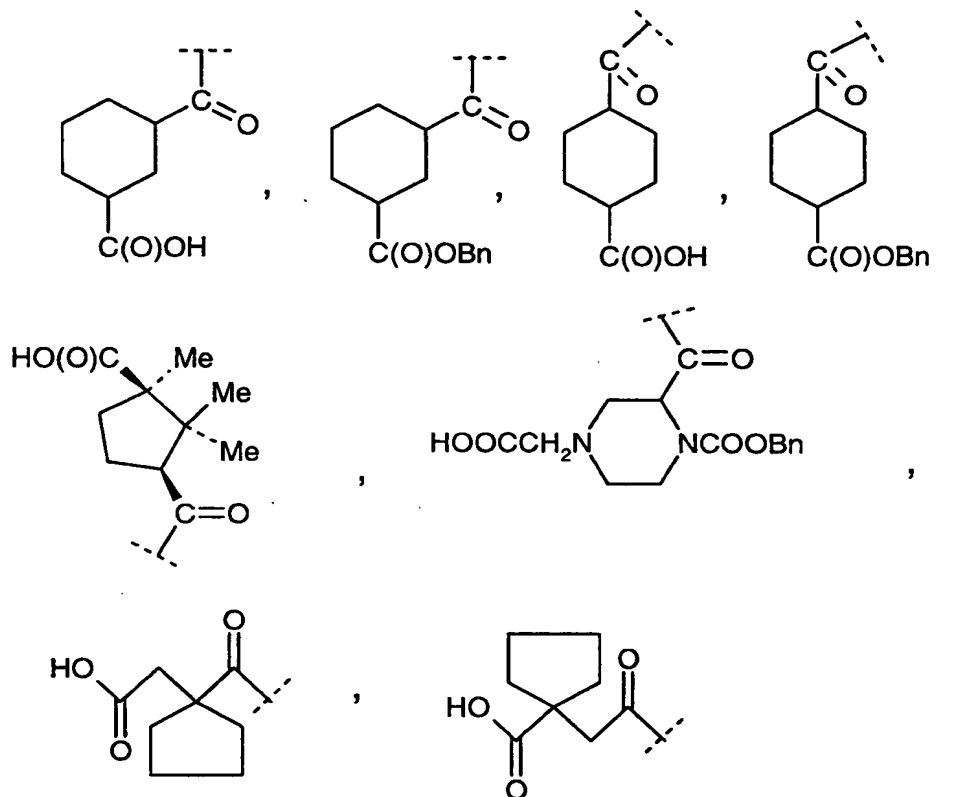W is a group of formula II':

15

Formula II'

wherein X is N; and $R_{2'}$ is as defined in claim 2, and

20   A is hydroxy or a pharmaceutically acceptable salt thereof; methoxy, ethoxy, phenoxy, or benzyloxy.

38.   A compound of formula Ia according to claim 2, wherein B is acetyl, 3-carboxypropionyl, 4-

25   carboxylbutyryl, $AcOCH_2C(O)$, $Me_3COC(O)$,

Y is H or Me, **a** is 0 or 1, **b** is 0 or 1,

R₆, when present, is the side chain of Asp or Glu,

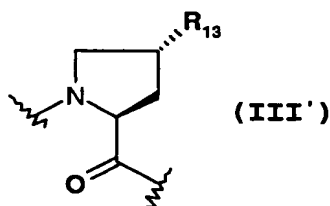**R₅**, when present, is the side chain of Asp, D-Asp, Glu, D-Glu, Val, D-Val or Tbg,

R₄ is the side chain of Val, Chg, Tbg, Ile or Leu,

R₃ is hydrogen or the side chain of Ile, Chg, Val, Glu;

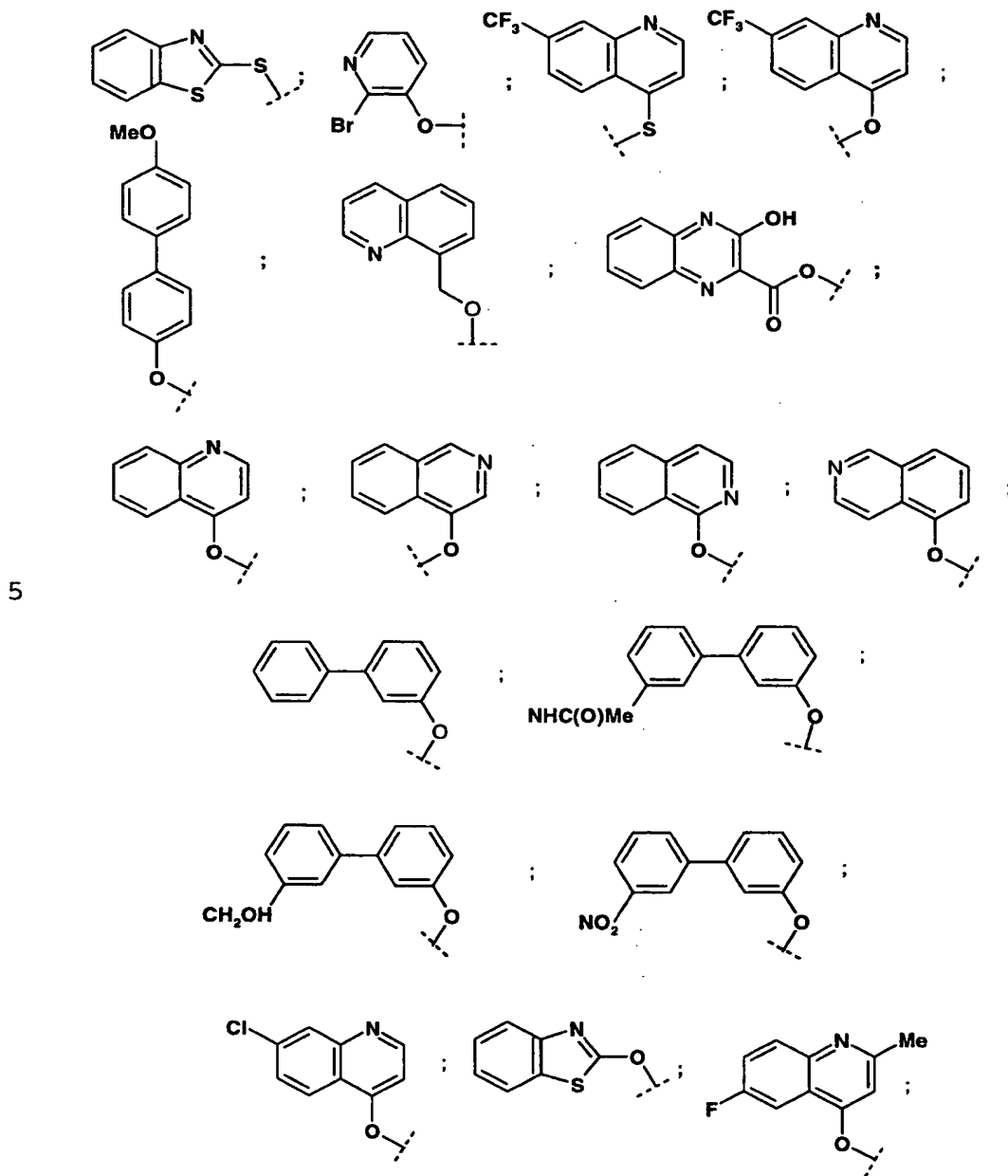**W** is Abu, Leu, Phe, Val, Ala, Glu, or Glu(OBn); or

W is group of formula III′:

(III′)

wherein **R₁₃** is Bn, PhCH₂CH₂, PhCH₂CH₂CH₂, O-Bn, o-tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-

naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-*tert*-butyl)benzyloxy, (3I-Ph)CH₂O, (4Br-Ph)O, (2Br-Ph)O, (3Br-Ph)O, (4I-Ph)O, (3Br-Ph)CH₂O, (3,5-Br₂-Ph)CH₂O,
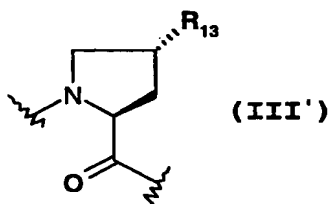

R₁, is H and R₁ is the side chain of Cys, Abu, Nva or allylglycine; or

$R_{1'}$ and R_1 together with the carbon atom to which they are attached form a cyclopropyl; and **A** is hydroxyl.
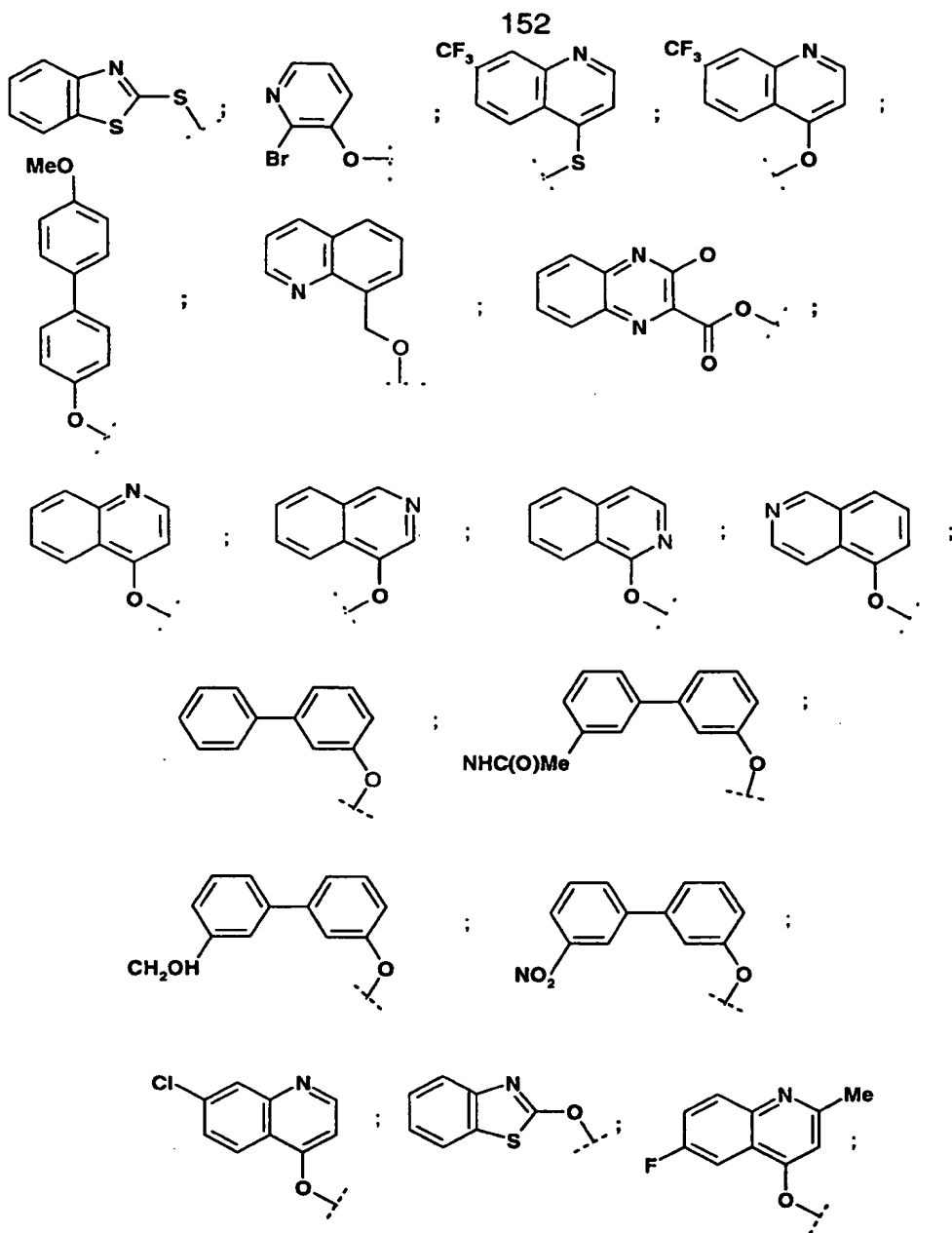
39. A compound of formula Ib according to claim 12, wherein and **B** is an amide of formula $R_{11a}N(R_{11b})-C(O)-$ wherein R_{11a} is C_{1-6} alkyl optionally substituted with carboxyl, C_{3-6} cycloalkyl, C_{3-7} (alkylcycloalkyl) optionally substituted with carboxy, $(C_{1-3}$ alkoxy)carbonyl, phenyl, C_{7-10} arylalkyl, 2-tetrahydrofuranylmethyl, or 2-thienylmethyl; and R_{11b} is $(C_{0-2}$ alkyl)phenyl optionally substituted with carboxyl or $(C_{1-4}$ alkoxy)carbonyl; or C_{1-6} alkyl substituted with carboxyl or $(C_{1-4}$ alkoxy)carbonyl; or R_{11a} and R_{11b} are joined to form a piperidine ring optionally substituted with carboxyl or $(C_{1-6}$ alkoxy)carbonyl;

R_4 is cyclohexyl,

Z is oxo;

R_3 is hydrogen or the side chain of Ile, Chg, Val, Glu;

W is Abu, Leu, Phe, Val, Ala, Glu, Glu(OBn); or

W is group of formula III':

(III')

wherein R_{13} is Bn, $PhCH_2CH_2$, $PhCH_2CH_2CH_2$, O-Bn, o-tolylmethoxy, m-tolylmethoxy, p-tolylmethoxy, 1-naphthalenylmethoxy, 2-naphthalenylmethoxy, (4-*tert*-butyl)methoxy, $(3I-Ph)CH_2O$, $(4Br-Ph)O$, $(2Br-Ph)O$, $(3Br-Ph)O$, $(4I-Ph)O$, $(3Br-Ph)CH_2O$, $(3,5-Br_2-Ph)CH_2O$,

30

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$R_{1'}$ is H and R_1 is the side chain of Cys, Abu, Nva or allylglycine; or

5      $R_{1'}$ and R_1 together with the carbon atom to which they are attached form a cyclopropyl; and A is hydroxyl.

40.   A compound of formula I according to claim 1, wherein B is an acyl derivative of formula $R_{11}-C(O)-$

wherein R_{11} is C_{1-10} alkyl optionally substituted with

carboxyl; C_{3-7} cycloalkyl optionally substituted with

carboxyl; or a C_{4-10} (alkylcycloalkyl) optionally

substituted on the cycloalkyl portion with carboxyl;

5   or R_{11} is C_6 or C_{10} aryl or C_{7-16} aralkyl optionally

substituted with a C_{1-6} alkyl

a is 0 or 1;

R_6, when present, is C_{1-6} alkyl optionally substituted

with carboxyl;

10   **b** is 0 or 1;

R_5, when present, is C_{1-6} alkyl optionally substituted

with carboxyl;

Q is N-**Y**, and **Y** is H or C_{1-6} alkyl;

R_4 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}

15   (alkylcycloalkyl);

Z is oxo,

R_3 is C_{1-10} alkyl, C_{3-7} cycloalkyl or C_{4-10}

(alkylcycloalkyl);

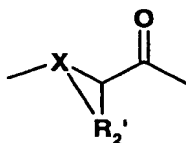**W** is a group of formula II:

20
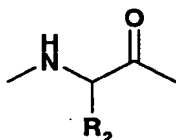
Formula II

wherein R_2 is C_{1-6} alkyl; C_{1-6} alkyl optionally

substituted with carboxyl; C_6 or C_{10} aryl; or C_{7-16}

25   aralkyl;

W is a group of formula II':

Formula II'

wherein **X** is CH or N; and

R₂′ is C_{3-4} alkyl that joins **X** to form a 5- or 6-
membered ring, said ring optionally substituted with

5   OH; SH; NH₂; carboxyl; **R₁₂**; OR₁₂, SR₁₂, NHR₁₂ or NR₁₂R₁₂′
wherein **R₁₂** and **R₁₂′** are independently:

cyclic C_{3-16} alkyl or acyclic C_{1-16} alkyl or
cyclic C_{3-16} alkenyl or acyclic C_{2-16} alkenyl,
said alkyl or alkenyl optionally substituted

10   with NH₂, OH, SH, halo, or carboxyl; said alkyl
or alkenyl optionally containing at least one
heteroatom selected independently from the group
consisting of: O, S, and N; or

R₁₂ and **R₁₂′** are independently C_6 or C_{10} aryl or

15   C_{7-16} aralkyl optionally substituted with C_{1-6}
alkyl, NH₂, OH, SH, halo, carboxyl or C_{1-6} alkyl
substituted with carboxyl; said aryl or aralkyl
optionally containing at least one heteroatom
selected independently from the group consisting

20   of: O, S, and N;
said cyclic alkyl, cyclic alkenyl, aryl or
aralkyl being optionally fused with a second 5-,
6-, or 7-membered ring to form a cyclic system
or heterocyclic system, said second ring being

25   optionally substituted with NH₂, OH, SH, halo,
carboxyl or carboxy(lower)alkyl; said second
ring optionally containing at least one
heteroatom selected independently from the group
consisting of: O, S, and N;

30   and

R₁′, is hydrogen, and **R₁** is C_{1-6} alkyl optionally
substituted with thiol, or C_{2-6} alkenyl; or
R₁′ and **R₁** together form a 3- to 6-membered ring
optionally substituted with C_{1-6} alkyl; and

A is hydroxyl or a pharmaceutically acceptable salt
or ester thereof.

41. A pharmaceutical composition comprising an anti-
5 hepatitis C virally effective amount of a compound of
formula I of claim 1, or a therapeutically acceptable
salt or ester thereof, in admixture with a
pharmaceutically acceptable carrier medium or
auxiliary agent.

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42. A method of treating a hepatitis C viral
infection in a mammal by administering to the mammal
an anti-hepatitis C virally effective amount of the
compound of formula I of claim 1, or a
15 therapeutically acceptable salt or ester thereof.

43. A method of inhibiting the replication of
hepatitis C virus by exposing the virus to a
hepatitis C viral NS3 protease inhibiting amount of
20 the compound of formula I of claim 1, or a
therapeutically acceptable salt or ester thereof.

44. A method of treating a hepatitis C viral
infection in a mammal by administering thereto an
25 anti-hepatitis C virally effective amount of a
combination of the compound of formula I of claim 1,
or a therapeutically acceptable salt or ester
thereof, and an interferon.

30 45. The use of a compound of formula I of claim 1
for the treatment of a hepatitis C infection in a
mammal comprising administering thereto an anti-
hepatitis C virally effective amount of the compound
of formula I.

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46.   The use of a compound of formula I of claim 1
for the manufacture of a mdeicament for treatment of
a hepatitis C infection in a mammal.

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